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(54) Title: HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS

(57) Abstract

The present invention describes human monoclonal antibodies which immunoreact with and neutralize human immunodeficiency virus (HIV). Also disclosed are immunotherapeutic and diagnostic methods of using the monoclonal antibodies, as well as cell line for producing the monoclonal antibodies.

HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES
TO HUMAN IMMUNODEFICIENCY VIRUS

Technical Field

5   The present invention relates generally to the
field of immunology and specifically to human
monoclonal antibodies which bind and neutralize human
immunodeficiency virus (HIV).

10   Background

1.   HIV Immunotherapy

HIV is the focus of intense studies as it is
the causative agent for acquired immunodeficiency
syndrome (AIDS). Immunotherapeutic methods are one of
15   several approaches to prevention, cure or remediation
of HIV infection and HIV-induced diseases.
Specifically, the use of neutralizing antibodies in
passive immunotherapies is of central importance to
the present invention.

20   Passive immunization of HIV-1 infected humans
using human sera containing polyclonal antibodies
immunoreactive with HIV has been reported. See for
example, Jackson et al., Lancet, September 17:647-652,
(1988); Karpas et al., Proc. Natl. Acad. Sci., USA,
25   87:7613-7616 (1990).

Numerous groups have reported the preparation of
human monoclonal antibodies that neutralize HIV
isolates in vitro. The described antibodies typically
have immunospecificities for epitopes on the HIV
30   glycoprotein gp120 or the related external surface
envelope glycoprotein gp120 or the transmembrane
glycoprotein gp41. See, for example Levy, Micro.
Rev., 57:183-289 (1993); Karwowska et al., Aids
Research and Human Retroviruses, 8:1099-1106 (1992);
35   Takeda et al., J. Clin. Invest., 89:1952-1957 (1992);

Tilley et al., <u>Aids Research and Human Retroviruses</u>,
8:461-467 (1992); Laman et al., <u>J. Virol.</u>, 66:1823-
1831 (1992); Thali et al., <u>J. Virol.</u>, 65:6188-6193
(1991); Ho et al., <u>Proc. Natl. Acad. Sci. USA</u>,
5 88:8949-8952 (1991); D'Souza et al., <u>AIDS</u>, 5:1061-
1070 (1991); Tilley et al., <u>Res. Virol.</u>, 142:247-259
(1991); Broliden et al., <u>Immunol.</u>, 73:371-376 (1991);
Matour et al., <u>J. Immunol.</u>, 146:4325-4332 (1991); and
Gorny et al., <u>Proc. Natl. Acad. Sci., USA</u>, 88:3238-
10 3242 (1991).

    To date, none of the reported human monoclonal
antibodies have been shown to be effective in passive
immunization therapies. Further, as monoclonal
antibodies, they all each react with an individual
15 epitope on the HIV envelope glycoprotein, gp120 or
gp160. The epitope against which an effective
neutralizing antibody immunoreacts has not been
identified.

    There continues to be a need to develop human
20 monoclonal antibody preparations with significant HIV
neutralization activity. In addition, there is a need
for monoclonal antibodies immunoreactive with
additional and diverse neutralizing epitopes on HIV
gp120 and gp41 in view of recent studies suggesting
25 that gp120 and gp41 are involved in both binding of
the HIV virus to the cell as well as in postbinding
events including envelope shedding and cleavage. See,
for review, Levy, <u>Micro. Rev.</u>, 57:183-289 (1993).
Additional (new) epitope specificities are required
30 because, upon passive immunization, the administered
patient can produce an immune response against the
administered antibody, thereby inactivating the
particular therapeutic antibody.

35   2. <u>Human Monoclonal Antibodies Produced From</u>

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Combinatorial Phagemid Libraries

The use of filamentous phage display vectors,
referred to as phagemids, has been repeatedly shown to
allow the efficient preparation of large libraries of
monoclonal antibodies having diverse and novel
immunospecificities. The technology uses a
filamentous phage coat protein membrane anchor domain
as a means for linking gene-product and gene during
the assembly stage of filamentous phage replication,
and has been used for the cloning and expression of
antibodies from combinatorial libraries. Kang et al.,
Proc. Natl. Acad. Sci., USA, 88:4363-4366 (1991).
Combinatorial libraries of antibodies have been
produced using both the cpVIII membrane anchor (Kang
et al., supra) and the cpIII membrane anchor. Barbas
et al., Proc. Natl. Acad. Sci., USA, 88:7978-7982
(1991).

The diversity of a filamentous phage-based
combinatorial antibody library can be increased by
shuffling of the heavy and light chain genes (Kang et
al., Proc. Natl. Acad. Sci., USA, 88:11120-11123
(1991)), by altering the CDR3 regions of the cloned
heavy chain genes of the library (Barbas et al., Proc.
Natl. Acad. Sci., USA, 89:4457-4461 (1992)), and by
introducing random mutations into the library by
error-prone polymerase chain reactions (PCR) [Gram et
al., Proc. Natl. Acad. Sci., USA, 89:3576-3580
(1992)].

Filamentous phage display vectors have also been
utilized to produce human monoclonal antibodies
immunoreactive with hepatitis B virus (HBV) or HIV
antigens. See, for example Zebedee et al., Proc.
Natl. Acad. Sci., USA, 89:3175-3179 (1992); and Burton
et al., Proc. Natl. Acad. Sci., USA, 88:10134-10137
(1991), respectively. None of the previously

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described human monoclonal antibodies produced by
phagemid vectors that are immunoreactive with HIV have
been shown to neutralize HIV.

5   Brief Description of the Invention
         Methods have now been discovered using the
phagemid vectors to identify and isolate from
combinatorial libraries human monoclonal antibodies
that neutralize HIV, and allow the rapid preparation
10   of large numbers of neutralizing antibodies of
completely human derivation. The identified
neutralizing antibodies define new epitopes on the HIV
gp120 and gp41 glycoproteins, thereby increasing the
availability of new immunotherapeutic human monoclonal
15   antibodies.
         The invention provides human monoclonal
antibodies that neutralize HIV, and also provides cell
lines used to produce these monoclonal antibodies.
         Also provided are amino acid sequences which
20   confer neutralization function to the antigen binding
domain of a monoclonal antibody, and which can be used
immunogenically to identify other antibodies that
specifically bind and neutralize HIV. The monoclonal
antibodies of the invention find particular utility as
25   reagents for the diagnosis and immunotherapy of HIV-
induced disease.
         A major advantage of the monoclonal antibodies of
the invention derives from the fact that they are
encoded by a human polynucleotide sequence. Thus, in
30   vivo use of the monoclonal antibodies of the invention
for diagnosis and immunotherapy of HIV-induced disease
greatly reduces the problems of significant host
immune response to the passively administered
antibodies which is a problem commonly encountered
35   when monoclonal antibodies of xenogeneic or chimeric

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derivation are utilized.

In one embodiment, the invention contemplates a human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp120 and neutralizing HIV. A preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 66, 67, 68, 70, 72, 73, 74, 75, 78 and 97. Another preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a light chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 95, 96, 97, 98, 101, 102, 103, 104, 105, 107, 110, 115, 118, 121, 122, 124 and 132.

In a further embodiment, the invention contemplates a human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp41 and neutralizing HIV. A preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 142, 143, 144, 145 and 146. Another preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a light chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 147, 148, 149, 150 and 151.

In another embodiment, the invention describes a polynucleotide sequence encoding a heavy or light chain immunoglobulin variable region amino acid residue sequence portion of a human monoclonal

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antibody of this invention. Also contemplated are DNA
expression vectors containing the polynucleotide, and
host cells containing the vectors and polynucleotides
of the invention.

5   The invention also contemplates a method of
detecting human immunodeficiency virus (HIV)
comprising contacting a sample suspected of containing
HIV with a diagnostically effective amount of the
monoclonal antibody of this invention, and determining
10   whether the monoclonal antibody immunoreacts with the
sample. The method can be practiced _in vitro_ or _in_
vivo, and may include a variety of methods for
determining the presence of an immunoreaction product.

           In another embodiment, the invention describes a
15   method for providing passive immunotherapy to human
immunodeficiency virus (HIV) disease in a human,
comprising administering to the human an
immunotherapeutically effective amount of the
monoclonal antibody of this invention. The
20   administration can be provided prophylactically, and
by a parenteral administration. Pharmaceutical
compositions containing one or more of the different
human monoclonal antibodies are described for use in
the therapeutic methods of the invention.

25

Brief Description of the Drawings

           In the drawings forming a portion of this
disclosure:

           Figure 1 illustrates the sequence of the
30   double-stranded synthetic DNA inserted into Lambda Zap
to produce a Lambda Hc2 expression vector. The
preparation of the double-stranded synthetic DNA
insert is described in Example 1a2). The various
features required for this vector to express the
35   V_H-coding DNA homologs include the Shine-Dalgarno

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ribosome binding site, a leader sequence to direct the
expressed protein to the periplasm as described by
Mouva et al., <u>J. Biol. Chem.</u>, 255:27, 1980, and
various restriction enzyme sites used to operatively
5   link the V_H homologs to the expression vector. The V_H
expression vector sequence also contains a short
nucleic acid sequence that codes for amino acids
typically found in variable regions heavy chain (V_H
backbone). This V_H backbone is just upstream and in
10   the proper reading as the V_H DNA homologs that are
operatively linked into the Xho I and Spe I cloning
sites. The sequences of the top and bottom strands of
the double-stranded synthetic DNA insert are listed
respectively in SEQ ID NO 1 and SEQ ID NO 2. The ten
15   amino acid sequence comprising the decapeptide tag is
listed in SEQ ID NO 5. The synthetic DNA insert is
directionally ligated into Lambda Zap II digested with
the restriction enzymes Not 1 and Xho I to form Lambda
Hc2 expression vector.

20   . Figure 2 illustrates the major features of the
bacterial expression vector Lambda Hc2 (V_H expression
vector). The orientation of the insert in Lambda Zap
II is shown. The V_H DNA homologs are inserted into
the Xho I and Spe I cloning sites. The read through
25   transcription produces the decapeptide epitope (tag)
that is located just 3' of the cloning site. The
amino acid residue sequence of the decapeptide tag and
the Pel B leader sequence/spacer are respectively
listed in SEQ ID NO 5 and 6.

30   Figure 3 illustrates the sequence of the double-
stranded synthetic DNA inserted into Lambda Zap to
produce a Lambda Lc2 expression vector. The various
features required for this vector to express the V_L-
coding DNA homologs are described in Figure 1. The
35   V_L-coding DNA homologs are operatively linked into the

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Lc2 sequence at the Sac I and Xho I restriction sites.
The sequences of the top and bottom strands of the
double-stranded synthetic DNA insert are listed
respectively in SEQ ID NO 3 and SEQ ID NO 4. The

5   synthetic DNA insert is directionally ligated into
Lambda Zap II digested with the restriction enzymes
Sac I and Not I to form Lambda Lc2 expression vector.
        Figure 4 illustrates the major features of the
bacterial expression vector Lc2 (V_L expression

10   vector). The synthetic DNA sequence from Figure 3 is
shown at the top along with the LacZ promoter from
Lambda Zap II. The orientation of the insert in
Lambda Zap II is shown. The V_L DNA homologs are
inserted into the Sac I and Xho I cloning sites. The

15   amino acid residue sequence of the Pel B leader
sequence/spacer is listed in SEQ ID NO 7.
        Figure 5 illustrates the dicistronic expression
vector, pComb, in the form of a phagemid expression
vector.

20   Figure 6 illustrates the neutralization of HIV-1
by recombinant Fabs. The same supernate preparations
were used in p24 and syncytia assays. The figures
indicate neutralization titers. Refer to Example 3
for details of the assay procedures and discussion of

25   the results. The ELISA titers and Fab concentrations
were determined as described in Example 2b.
        Figure 7 illustrates the relative affinities of
Fab fragments for gp120 (IIIB) as illustrated by
inhibition ELISA performed as described in Example

30   2b6). Fabs 27, 6, 29, 2 and 3 are all prototype
members of the different groups discussed in Example
4. Loop 2 is an Fab fragment selected from the same
library as the other Fabs but which recognizes the V3
loop. The data is plotted as the percentage of

35   maximum binding on the Y-axis against increasing

concentrations (10^{-11} M to 10^{-7} M) of soluble gp120 on the X-axis.

Figure 8 illustrates the soluble CD4 competition with Fab fragments for gp120 (IIIB). P4D10 and loop2 are controls. P4D10 is a mouse monoclonal antibody reacting with the V3 loop of gp120 (IIIB). The data, discussed in Example 2b6), is plotted as described in Figure 7.

Figure 9 illustrates the neutralization of HIV by purified Fabs prepared as described in Example 3. The results shown are derived from the syncytia assay using the MN strain. The data is plotted as percent of inhibition of binding on the Y-axis against increasing Fab concentrations [0.1 to greater than 10 micrograms/milliliter (μg/ml)] on the X-axis.

Figure 10 illustrates the amino acid residue sequences of variable heavy (V_H) domains of Fabs binding to gp120. Seven distinct groups have been identified as described in Example 4 based on sequence homology. Identity with the first sequence in a group is indicated by dots. The Fab clone names are indicated in the left hand column. The corresponding SEQ ID NOs are indicated in the right hand column. The sequenced regions from right to left are framework region 1 (FR1), complementary determining region 1 (CDR1), framework region 2 (FR2), complementary determining region 2 (CDR2), framework region 3 (FR3), complementary determining region 3 (CDR3), and framework region 4 (FR4). The five amino-terminal residue sequence beginning with LEQ arises from the VH1a while the 5 amino-terminal residue sequence beginning with LEE arises from the VH3a primers. The b11 and b29 sequences are very similar to the b3 group and could be argued to be intraclonal variants within that group; they are placed in their own group because

of differences at the V-D and D-J interface.

Figure 11 illustrates the amino acid residue
sequences of variable light (V_L) domains of Fabs
binding to gp120. Refer to Figure 10 for the
description of the figure and to Example 4 for
analysis of the sequences.

Figure 12 illustrates the amino acid residue
sequences of V_L domains from Fabs binding to gp120 and
generated by shuffling the heavy chain from clone b12
against a library of light chains (H12-LCn Fabs) as
described in Example 5. Note that the new V_L
sequences have designated clone numbers that do not
relate to those numbers from the original library.
The unique sequences are listed in the Sequence
Listing from SEQ ID NO 114 to 122. The new V_L domain
sequences are compared to that of the original clone
b12 V_L sequence.

Figure 13 illustrates the amino acid residue
sequences of V_H domains from Fabs binding to gp120 and
generated by shuffling the light chain from clone b12
against a library of heavy chains (L12-HCn Fabs) as
described in Example 5. Note that the new V_H
sequences have designated clone numbers that do not
relate to those numbers from the original library.
The unique sequences are listed in the Sequence
Listing from SEQ ID NO 123 to 132. The new V_H domain
sequences are compared to that of the original clone
b12 V_H sequence.

Figure 14 illustrates, in two figures, Figure 14A
and 14B, plasmid maps of the heavy (pTAC01H) and light
chain (pTC01) replicon-compatible chain-shuffling
vectors, respectively. Both plasmids are very similar
in the section containing the promoter and the cloning
site. Abbreviations: tacPO, tac promoter/operon; 5
histidine amino acid residue tag (histidine)5-tail;

f1IG, intergenic region of f1-phage; stu, stuffer fragment ready for in-frame replacement by light and heavy chain, respectively; cat, chloramphenicol transferase gene; bla, b-lactamase gene; ori, origin of replication. The map is drawn approximately to scale.

Figure 15 illustrates the nucleotide sequences of the binary shuffling vectors in two Figures, 15A and 15B. The construction and use of the vectors is described in Example 6. In Figure 15A, the double-stranded nucleotide sequence of the multiple cloning site in light chain vector, pTC01, is shown. The sequences of the top and bottom nucleotide base strands are listed respectively in SEQ ID NO 8 and SEQ ID NO 9. The amino acid residue sequence comprising the pelB leader ending in the Sac I restriction site is listed in SEQ ID NO 10. In Figure 15B, the nucleotide sequence of the multiple cloning site in heavy chain vector, pTAC01H, is shown. The sequences of the top and bottom nucleotide base strands are listed respectively in SEQ ID NO 11 and SEQ ID NO 12. The amino acid residue sequence comprising the pelB leader ending in the Xho I restriction site is listed as SEQ ID NO 13. The amino acid residue sequence comprising the histidine tail is listed in SEQ ID NO 14. Relevant restriction sites are underlined. tac promoter and ribosome binding site (rbs) are indicated by boxes.

Figure 16 illustrates the complete set of directed crosses between heavy and light chains of all Fab fragments isolated from the original library by panning with gp160 (IIIB) (b1-b27), gp120 (IIIB) (B8-B35), gp120 (SF2) (s4-s8), and the loop peptide (p35) assayed by ELISA against IIIB gp120 as described in Example 6. Heavy chains are listed horizontally

and light chains are listed vertically. Clones are
sorted according to the grouping established in
Example 4. Different groups are separated by
horizontal and vertical lines. A "-" at the
5   intersection of a particular heavy chain and light
chain signifies a clear negative (a signal of 3 times
background or less) for that particular cross, a "+"
shows a clear positive comparable to the original
heavy and light chain combination, and a "w" denotes
10   an intermediate value in the ELISA. "●": the HCp35/
LCp35 combination is negative when gp120 (IIIB) is
used, but positive when assayed with gp120 (IIIB).
Identical chains carry the same identifier (either *,
¶, §, or ¥).
15   Figure 17 illustrates the affinity of
antibody-antigen interaction for b12 heavy chain
crosses with light chains from all pannings analyzed
by competitive ELISA using soluble IIIB gp120 as
competing antigen as described in Example 6. The data
20   is plotted as the percentage of maximum binding on the
Y-axis against increasing concentrations of soluble
gp120 (IIIB) (10^{-12} M to 10^{-7} M) on the X-axis.
Figure 18 illustrates the amino acid residue
sequences of variable heavy (V_H) domains of Fabs
25   binding to gp41. The Fab clone names are indicated in
the left hand column. The heavy chain sequences of
the five Fabs individually designated DL 41 19, DO 41
11, GL 41 1, MT 41 12 and SS 41 8 have been assigned
the respective SEQ ID NOs 142, 143, 144, 145 and 146.
30   The sequenced regions from right to left are framework
region 1 (FR1), complementary determining region 1
(CDR1), framework region 2 (FR2), complementary
determining region 2 (CDR2), framework region 3 (FR3),
complementary determining region 3 (CDR3), and
35   framework region 4 (FR4).

Figure 19 illustrates the amino acid residue
sequences of variable light (V_L) domains of Fabs
binding to gp41. Refer to Figure 18 for the
description of the figure. The light chain sequences
5   of the five Fabs individually designated DL 41 19, DO
41 11, GL 41 1, MT 41 12 and SS 41 8 have been
assigned the respective SEQ ID NOs 147, 148, 149, 150
and 151.

10   Detailed Description of the Invention
        A.   Definitions
            Amino Acid Residue: An amino acid formed
upon chemical digestion (hydrolysis) of a polypeptide
at its peptide linkages. The amino acid residues
15   described herein are preferably in the "L" isomeric
form. However, residues in the "D" isomeric form can
be substituted for any L-amino acid residue, as long
as the desired functional property is retained by the
polypeptide. NH_2 refers to the free amino group
20   present at the amino terminus of a polypeptide. COOH
refers to the free carboxy group present at the
carboxy terminus of a polypeptide. In keeping with
standard polypeptide nomenclature (described in J.
Biol. Chem., 243:3552-59 (1969) and adopted at 37 CFR
25   §1.822(b)(2)), abbreviations for amino acid residues
are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

	SYMBOL		AMINO ACID
	1-Letter	3-Letter	
	Y	Tyr	tyrosine
	G	Gly	glycine
	F	Phe	phenylalanine
	M	Met	methionine
	A	Ala	alanine

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S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

It should be noted that all amino acid residue
sequences represented herein by formulae have a left-
to-right orientation in the conventional direction of
amino terminus to carboxy terminus. In addition, the
phrase "amino acid residue" is broadly defined to
include the amino acids listed in the Table of
Correspondence and modified and unusual amino acids,
such as those listed in 37 CFR 1.822(b)(4), and
incorporated herein by reference. Furthermore, it
should be noted that a dash at the beginning or end of
an amino acid residue sequence indicates a peptide
bond to a further sequence of one or more amino acid
residues or a covalent bond to an amino-terminal group
such as NH_2 or acetyl or to a carboxy-terminal group
such as COOH.

Recombinant DNA (rDNA) molecule: A DNA molecule
produced by operatively linking two DNA segments.

Thus, a recombinant DNA molecule is a hybrid DNA
molecule comprising at least two nucleotide sequences
not normally found together in nature. rDNA's not
having a common biological origin, i.e.,
5   evolutionarily different, are said to be
"heterologous".

       Vector: A rDNA molecule capable of autonomous
replication in a cell and to which a DNA segment,
e.g., gene or polynucleotide, can be operatively
10   linked so as to bring about replication of the
attached segment. Vectors capable of directing the
expression of genes encoding for one or more
polypeptides are referred to herein as "expression
vectors". Particularly important vectors allow
15   cloning of cDNA (complementary DNA) from mRNAs
produced using reverse transcriptase.

       Receptor: A receptor is a molecule, such as a
protein, glycoprotein and the like, that can
specifically (non-randomly) bind to another molecule.
20   Antibody: The term antibody in its various
grammatical forms is used herein to refer to
immunoglobulin molecules and immunologically active
portions of immunoglobulin molecules, i.e., molecules
that contain an antibody combining site or paratope.
25   Exemplary antibody molecules are intact immunoglobulin
molecules, substantially intact immunoglobulin
molecules and portions of an immunoglobulin molecule,
including those portions known in the art as Fab,
Fab', F(ab')$_2$ and F(v).

30   Antibody Combining Site: An antibody combining
site is that structural portion of an antibody
molecule comprised of a heavy and light chain variable
and hypervariable regions that specifically binds
(immunoreacts with) an antigen. The term immunoreact
35   in its various forms means specific binding between an

antigenic determinant-containing molecule and a
molecule containing an antibody combining site such as
a whole antibody molecule or a portion thereof.

Monoclonal Antibody: A monoclonal antibody in
its various grammatical forms refers to a population
of antibody molecules that contain only one species of
antibody combining site capable of immunoreacting with
a particular epitope. A monoclonal antibody thus
typically displays a single binding affinity for any
epitope with which it immunoreacts. A monoclonal
antibody may therefore contain an antibody molecule
having a plurality of antibody combining sites, each
immunospecific for a different epitope, e.g., a
bispecific monoclonal antibody. Although historically
a monoclonal antibody was produced by immortalization
of a clonally pure immunoglobulin secreting cell line,
a monoclonally pure population of antibody molecules
can also be prepared by the methods of the present
invention.

Fusion Polypeptide: A polypeptide comprised of
at least two polypeptides and a linking sequence to
operatively link the two polypeptides into one
continuous polypeptide. The two polypeptides linked
in a fusion polypeptide are typically derived from two
independent sources, and therefore a fusion
polypeptide comprises two linked polypeptides not
normally found linked in nature.

Upstream: In the direction opposite to the
direction of DNA transcription, and therefore going
from 5' to 3' on the non-coding strand, or 3' to 5' on
the mRNA.

Downstream: Further along a DNA sequence in the
direction of sequence transcription or read out, that
is traveling in a 3'- to 5'-direction along the non-
coding strand of the DNA or 5'- to 3'-direction along

the RNA transcript.

Cistron: Sequence of nucleotides in a DNA
molecule coding for an amino acid residue sequence and
including upstream and downstream DNA expression
5   control elements.

Leader Polypeptide: A short length of amino acid
sequence at the amino end of a polypeptide, which
carries or directs the polypeptide through the inner
membrane and so ensures its eventual secretion into
10   the periplasmic space and perhaps beyond. The leader
sequence peptide is commonly removed before the
polypeptide becomes active.

Reading Frame: Particular sequence of contiguous
nucleotide triplets (codons) employed in translation.
15   The reading frame depends on the location of the
translation initiation codon.

B.   Human Monoclonal Antibodies

The present invention relates to human mono-
20   clonal antibodies which are specific for, and
neutralize human immunodeficiency virus (HIV). In a
preferred embodiment of the invention, human
monoclonal antibodies are disclosed which are capable
of binding epitopic polypeptide sequences in
25   glycoprotein gp120 of HIV. A further preferred
embodiment are human monoclonal antibodies capable of
binding epitopic polypeptide sequences in glycoprotein
gp 41 of HIV. Also disclosed is an antibody having a
specified amino acid sequence, which sequence confers
30   the ability to bind a specific epitope and to
neutralize HIV when the virus is bound by these
antibodies. A human monoclonal antibody with a
claimed specificity, and like human monoclonal
antibodies with like specificity, are useful in the
35   diagnosis and immunotherapy of HIV-induced disease.

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The term "HIV-induced disease" means any disease caused, directly or indirectly, by HIV. An example of a HIV-induced disease is acquired autoimmunodeficiency syndrome (AIDS), and any of the numerous conditions

5   associated generally with AIDS which are caused by HIV infection.

Thus, in one aspect, the present invention is directed to human monoclonal antibodies which are reactive with a HIV neutralization site and cell lines

10   which produce such antibodies. The isolation of cell lines producing monoclonal antibodies of the invention is described in great detail further herein, and can be accomplished using the phagemid vector library methods described herein, and using routine screening

15   techniques which permit determination of the elementary immunoreaction and neutralization patterns of the monoclonal antibody of interest. Thus, if a human monoclonal antibody being tested binds and neutralizes HIV in a manner similar to a human

20   monoclonal antibody produced by the cell lines of the invention then the tested antibody is considered equivalent to an antibody of the invention.

It is also possible to determine, without undue experimentation, if a human monoclonal antibody has

25   the same (i.e., equivalent) specificity as a human monoclonal antibody of this invention by ascertaining whether the former prevents the latter from binding to HIV. If the human monoclonal antibody being tested competes with the human monoclonal antibody of the

30   invention, as shown by a decrease in binding by the human monoclonal antibody of the invention in standard competition assays for binding to a solid phase antigen, for example to gp120, then it is likely that the two monoclonal antibodies bind to the same, or a

35   closely related, epitope.

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Still another way to determine whether a human
monoclonal antibody has the specificity of a human
monoclonal antibody of the invention is to pre-
incubate the human monoclonal antibody of the
5   invention with HIV with which it is normally reactive,
and then add the human monoclonal antibody being
tested to determine if the human monoclonal antibody
being tested is inhibited in its ability to bind HIV.
If the human monoclonal antibody being tested is
10   inhibited then, in all likelihood, it has the same, or
functionally equivalent, epitopic specificity as the
monoclonal antibody of the invention. Screening of
human monoclonal antibodies of the invention, can be
also carried out utilizing HIV neutralization assays
15   and determining whether the monoclonal antibody
neutralizes HIV.

The ability to neutralize HIV at one or more
stages of virus infection is a desirable quality of a
human monoclonal antibody of the present invention.
20   Virus neutralization can be measured by a variety of
in vitro and in vivo methodologies. Exemplary methods
described herein for determining the capacity for
neutralization are the in vitro assays that measure
inhibition of HIV-induced syncytia formation, plaque
25   assays and assays that measure the inhibition of
output of core p24 antigen from a cell infected with
HIV.

As shown herein, the immunospecificity of a human
monoclonal antibody of this invention can be directed
30   to epitopes that are shared across serotypes and/or
strains of HIV, or can be specific for a single strain
of HIV, depending upon the epitope. Thus, a preferred
human monoclonal antibody can immunoreact with HIV-1,
HIV-2, or both, and can immunoreact with one or more
35   of the HIV-1 strains IIIB, MN, RF, SF-2, Z2, Z6, CDC4,

ELI and the like strains.

The immunospecificity of an antibody, its HIV-
neutralizing capacity, and the attendant affinity the
antibody exhibits for the epitope, are defined by the
epitope with which the antibody immunoreacts. The
epitope specificity is defined at least in part by the
amino acid residue sequence of the variable region of
the heavy chain of the immunoglobulin the antibody,
and in part by the light chain variable region amino
acid residue sequence. Preferred human monoclonal
antibodies immunoreact with the CD4 binding site of
glycoprotein gp120.

A preferred human monoclonal antibody of this
invention has the binding specificity of a monoclonal
antibody comprising a heavy chain immunoglobulin
variable region amino acid residue sequence selected
from the group of sequences consisting of SEQ ID NOs
66, 67, 68, 70, 72, 73, 74, 75, 78 and 97, and
conservative substitutions thereof.

Another preferred human monoclonal antibody of
this invention has the binding specificity of a
monoclonal antibody having a light chain
immunoglobulin variable region amino acid residue
sequence selected from the group of sequences
consisting of SEQ ID NOs 95, 96, 97, 98, 101, 102,
103, 104, 105, 107, 110, 115, 118, 121, 122, 124 and
132, and conservative substitutions thereof.

Further preferred human monoclonal antibodies
immunoreact with the CD4 binding site of glycoprotein
gp41. A preferred human monoclonal antibody of this
invention has the binding specificity of a monoclonal
antibody comprising a heavy chain immunoglobulin
variable region amino acid residue sequence selected
from the group of sequences consisting of SEQ ID NOs
142, 143, 144, 145, and 146 and conservative

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substitutions thereof.

Another preferred human monoclonal antibody of
this invention has the gp41 binding specificity of a
monoclonal antibody having a light chain
5   immunoglobulin variable region amino acid residue
sequence selected from the group of sequences
consisting of SEQ ID NOs 147, 148, 149, 150, and 151
and conservative substitutions thereof.

As shown by the present teachings and using the
10   combinatorial library shuffling and screening methods,
one can identify new heavy and light chain pairs (H:L)
that function as a HIV-neutralizing monoclonal
antibody. In particular, one can shuffle a known
heavy chain, derived from an HIV-neutralizing human
15   monoclonal antibody, with a library of light chains to
identify new H:L pairs that form a functional antibody
according to the present invention. Similarly, one
can shuffle a known light chain, derived from an HIV-
neutralizing human monoclonal antibody, with a library
20   of heavy chains to identify new H:L pairs that form a
functional antibody according to the present
invention.

Particularly preferred human monoclonal
antibodies are those having the gp120 immunoreaction
25   (binding) specificity of a monoclonal antibody having
heavy and light chain immunoglobulin variable region
amino acid residue sequences in pairs (H:L) selected
from the group consisting of SEQ ID NOs 66:95, 67:96,
72:102, 66:97, 73:107, 74:103, 70:101, 68:98, 75:104,
30   72:105, 78:110, 66:118, 66:122, 66:121, 66:115,
97:124, 97:132 and 66:98, and conservative
substitutions thereof. The designation of two SEQ ID
NOs with a colon, e.g., 66:95, is to connote a H:L
pair formed by the heavy and light chain,
35   respectively, amino acid residue sequences shown in

SEQ ID NO 66 and SEQ ID NO 95, respectively.

Further preferred human monoclonal antibodies are
those having the gp41 immunoreaction (binding)
specificity of a monoclonal antibody having heavy and
5   light chain immunoglobulin variable region amino acid
residue sequences in pairs (H:L) selected from the
group consisting of SEQ ID NOs 142:147, 143:148,
144:149, 145:150, and 146:151, and conservative
substitutions thereof.

10   Particularly preferred are human monoclonal
antibodies having the binding specificity of the
monoclonal antibody produced by the E. coli
microorganisms deposited with the ATCC, as described
further herein.

15

Particularly preferred are human monoclonal
antibodies having the binding specificity of the
monoclonal antibodies produced by the E. coli
microorganisms designated ATCC 69078, 69079 and 69080.
20   By "having the binding specificity" is meant
equivalent monoclonal antibodies which exhibit the
same or similar immunoreaction and neutralization
properties, and which compete for binding to an HIV
antigen. Preferred are the human monoclonal
25   antibodies produced by ATCC 69078, 69079 and 69080.

The term "conservative variation" as used herein
denotes the replacement of an amino acid residue by
another, biologically similar residue. Examples of
conservative variations include the substitution of
30   one hydrophobic residue such as isoleucine, valine,
leucine or methionine for another, or the substitution
of one polar residue for another, such as the
substitution of arginine for lysine, glutamic for
aspartic acids, or glutamine for asparagine, and the
35   like. The term "conservative variation" also includes

the use of a substituted amino acid in place of an
unsubstituted parent amino acid provided that
antibodies having the substituted polypeptide also
neutralize HIV. Analogously, another preferred

5   embodiment of the invention relates to polynucleotides
which encode the above noted heavy and/or light chain
polypeptides and to polynucleotide sequences which are
complementary to these polynucleotide sequences.
Complementary polynucleotide sequences include those

10   sequences which hybridize to the polynucleotide
sequences of the invention under stringent
hybridization conditions.

By using the human monoclonal antibodies of the
invention, it is now possible to produce anti-

15   idiotypic antibodies which can be used to screen human
monoclonal antibodies to identify whether the antibody
has the same binding specificity as a human monoclonal
antibody of the invention and also used for active
immunization (Herlyn et al., Science, 232:100 (1986)).

20   Such anti-idiotypic antibodies can be produced using
well-known hybridoma techniques (Kohler et al.,
Nature, 256:495 (1975)). An anti-idiotypic antibody
is an antibody which recognizes unique determinants
present on the human monoclonal antibody produced by

25   the cell line of interest. These determinants are
located in the hypervariable region of the antibody.
It is this region which binds to a given epitope and,
thus, is responsible for the specificity of the
antibody. An anti-idiotypic antibody can be prepared

30   by immunizing an animal with the monoclonal antibody
of interest. The immunized animal will recognize and
respond to the idiotypic determinants of the
immunizing antibody and produce an antibody to these
idiotypic determinants. By using the anti-idiotypic

35   antibodies of the immunized animal, which are specific

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for the human monoclonal antibody of the invention
produced by a cell line which was used to immunize the
second animal, it is now possible to identify other
clones with the same idiotype as the antibody of the
5   hybridoma used for immunization. Idiotypic identity
between human monoclonal antibodies of two cell lines
demonstrates that the two monoclonal antibodies are
the same with respect to their recognition of the same
epitopic determinant. Thus, by using anti-idiotypic
10   antibodies, it is possible to identify other
hybridomas expressing monoclonal antibodies having the
same epitopic specificity.

It is also possible to use the anti-idiotype
technology to produce monoclonal antibodies which
15   mimic an epitope. For example, an anti-idiotypic
monoclonal antibody made to a first monoclonal
antibody will have a binding domain in the
hypervariable region which is the "image" of the
epitope bound by the first monoclonal antibody. Thus,
20   the anti-idiotypic monoclonal antibody can be used for
immunization, since the anti-idiotype monoclonal
antibody binding domain effectively acts as an
antigen.

In one preferred embodiment, the invention
25   contemplates a truncated immunoglobulin molecule
comprising a Fab fragment derived from a human
monoclonal antibody of this invention. The Fab
fragment, lacking Fc receptor, is soluble, and affords
therapeutic advantages in serum half life, and
30   diagnostic advantages in modes of using the soluble
Fab fragment. The preparation of a soluble Fab
fragment is generally known in the immunological arts
and can be accomplished by a variety of methods. A
preferred method of producing a soluble Fab fragment
35   is described herein.

C.   Immunotherapeutic Methods and Compositions

The human monoclonal antibodies can also be
used immunotherapeutically for HIV disease. The term
"immunotherapeutically" or "immunotherapy" as used
5   herein in conjunction with the monoclonal antibodies
of the invention denotes both prophylactic as well as
therapeutic administration. Thus, the monoclonal
antibodies can be administered to high-risk patients
in order to lessen the likelihood and/or severity of
10   HIV-induced disease, administered to patients already
evidencing active HIV infection, or administered to
patients at risk of HIV infection.

1.   Therapeutic Compositions

15   The present invention therefore
contemplates therapeutic compositions useful for
practicing the therapeutic methods described herein.
Therapeutic compositions of the present invention
contain a physiologically tolerable carrier together
20   with at least one species of human monoclonal antibody
as described herein, dissolved or dispersed therein as
an active ingredient. In a preferred embodiment, the
therapeutic composition is not immunogenic when
administered to a human patient for therapeutic
25   purposes, unless that purpose is to induce an immune
response, as described elsewhere herein.

As used herein, the terms "pharmaceutically
acceptable", "physiologically tolerable" and
grammatical variations thereof, as they refer to
30   compositions, carriers, diluents and reagents, are
used interchangeably and represent that the materials
are capable of administration to or upon a human
without the production of undesirable physiological
effects such as nausea, dizziness, gastric upset and
35   the like.

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The preparation of a pharmacological composition
that contains active ingredients dissolved or
dispersed therein is well understood in the art.
Typically such compositions are prepared as sterile
5   injectables either as liquid solutions or suspensions,
aqueous or non-aqueous, however, solid forms suitable
for solution, or suspensions, in liquid prior to use
can also be prepared. The preparation can also be
emulsified.

10   The active ingredient can be mixed with
excipients which are pharmaceutically acceptable and
compatible with the active ingredient and in amounts
suitable for use in the therapeutic methods described
herein. Suitable excipients are, for example, water,
15   saline, dextrose, glycerol, ethanol or the like and
combinations thereof. In addition, if desired, the
composition can contain minor amounts of auxiliary
substances such as wetting or emulsifying agents, pH
buffering agents and the like which enhance the
20   effectiveness of the active ingredient.

The therapeutic composition of the present
invention can include pharmaceutically acceptable
salts of the components therein. Pharmaceutically
acceptable salts include the acid addition salts
25   (formed with the free amino groups of the polypeptide)
that are formed with inorganic acids such as, for
example, hydrochloric or phosphoric acids, or such
organic acids as acetic, tartaric, mandelic and the
like. Salts formed with the free carboxyl groups can
30   also be derived from inorganic bases such as, for
example, sodium, potassium, ammonium, calcium or
ferric hydroxides, and such organic bases as
isopropylamine, trimethylamine, 2-ethylamino ethanol,
histidine, procaine and the like.
35   Physiologically tolerable carriers are well known

in the art. Exemplary of liquid carriers are sterile
aqueous solutions that contain no materials in
addition to the active ingredients and water, or
contain a buffer such as sodium phosphate at

5   physiological pH value, physiological saline or both,
such as phosphate-buffered saline. Still further,
aqueous carriers can contain more than one buffer
salt, as well as salts such as sodium and potassium
chlorides, dextrose, propylene glycol, polyethylene

10   glycol and other solutes.

Liquid compositions can also contain liquid
phases in addition to and to the exclusion of water.
Exemplary of such additional liquid phases are
glycerin, vegetable oils such as cottonseed oil,

15   organic esters such as ethyl oleate, and water-oil
emulsions.

A therapeutic composition contains an HIV-
neutralizing of a human monoclonal antibody of the
present invention, typically an amount of at least 0.1

20   weight percent of antibody per weight of total
therapeutic composition. A weight percent is a ratio
by weight of antibody to total composition. Thus, for
example, 0.1 weight percent is 0.1 grams of antibody
per 100 grams of total composition.

25

2.   Therapeutic Methods

In view of the demonstrated HIV
neutralizing ability of the human monoclonal
antibodies of the present invention, the present

30   disclosure provides for a method for neutralizing HIV
in vitro or in vivo. The method comprises contacting
a sample believed to contain HIV with a composition
comprising a therapeutically effective amount of a
human monoclonal antibody of this invention.

35   For in vivo modalities, the method comprises

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administering to the patient a therapeutically
effective amount of a physiologically tolerable
composition containing a human monoclonal antibody of
the invention. Thus, the present invention describes
5   in one embodiment a method for providing passive
immunotherapy to HIV disease in a human comprising
administering to the human an immunotherapeutically
effective amount of the monoclonal antibody of this
invention.

10   A representative patient for practicing the
present passive immunotherapeutic methods is any human
exhibiting symptoms of HIV-induced disease, including
AIDS or related conditions believed to be caused by
HIV infection, and humans at risk of HIV infection.
15   Patients at risk of infection by HIV include babies of
HIV-infected pregnant mothers, recipients of
transfusions known to contain HIV, users of HIV
contaminated needles, individuals who have
participated in high risk sexual activities with known
20   HIV-infected individuals, and the like risk
situations.

In one embodiment, the passive immunization
method comprises administering a composition
comprising more than one species of human monoclonal
25   antibody of this invention, preferably directed to
non-competing epitopes or directed to distinct
serotypes or strains of HIV, as to afford increased
effectiveness of the passive immunotherapy.

A therapeutically (immunotherapeutically)
30   effective amount of a human monoclonal antibody is a
predetermined amount calculated to achieve the desired
effect, i.e., to neutralize the HIV present in the
sample or in the patient, and thereby decrease the
amount of detectable HIV in the sample or patient. In
35   the case of _in vivo_ therapies, an effective amount can

be measured by improvements in one or more symptoms
associated with HIV-induced disease occurring in the
patient, or by serological decreases in HIV antigens.

5   Thus, the dosage ranges for the administration of
the monoclonal antibodies of the invention are those
large enough to produce the desired effect in which
the symptoms of the HIV disease are ameliorated or the
likelihood of infection decreased. The dosage should
not be so large as to cause adverse side effects, such

10   as hyperviscosity syndromes, pulmonary edema,
congestive heart failure, and the like. Generally,
the dosage will vary with the age, condition, sex and
extent of the disease in the patient and can be
determined by one of skill in the art.

15   The dosage can be adjusted by the individual
physician in the event of any complication.

          A therapeutically effective amount of an antibody
of this invention is typically an amount of antibody
such that when administered in a physiologically

20   tolerable composition is sufficient to achieve a
plasma concentration of from about 0.1 microgram (ug)
per milliliter (ml) to about 100 ug/ml, preferably
from about 1 ug/ml to about 5 ug/ml, and usually about
5 ug/ml. Stated differently, the dosage can vary from

25   about 0.1 mg/kg to about 300 mg/kg, preferably from
about 0.2 mg/kg to about 200 mg/kg, most preferably
from about 0.5 mg/kg to about 20 mg/kg, in one or more
dose administrations daily, for one or several days.

          The human monoclonal antibodies of the invention

30   can be administered parenterally by injection or by
gradual infusion over time. Although the HIV
infection is typically systemic and therefore most
often treated by intravenous administration of
therapeutic compositions, other tissues and delivery

35   means are contemplated where there is a likelihood

that the tissue targeted contains infectious HIV.
Thus, human monoclonal antibodies of the invention can
be administered intravenously, intraperitoneally,
intramuscularly, subcutaneously, intracavity,
5   transdermally, and can be delivered by peristaltic
means.

The therapeutic compositions containing a human
monoclonal antibody of this invention are
conventionally administered intravenously, as by
10   injection of a unit dose, for example. The term "unit
dose" when used in reference to a therapeutic
composition of the present invention refers to
physically discrete units suitable as unitary dosage
for the subject, each unit containing a predetermined
15   quantity of active material calculated to produce the
desired therapeutic effect in association with the
required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner
compatible with the dosage formulation, and in a
20   therapeutically effective amount. The quantity to be
administered depends on the subject to be treated,
capacity of the subject's system to utilize the active
ingredient, and degree of therapeutic effect desired.
Precise amounts of active ingredient required to be
25   administered depend on the judgement of the
practitioner and are peculiar to each individual.
However, suitable dosage ranges for systemic
application are disclosed herein and depend on the
route of administration. Suitable regimes for
30   administration are also variable, but are typified by
an initial administration followed by repeated doses
at one or more hour intervals by a subsequent
injection or other administration. Alternatively,
continuous intravenous infusion sufficient to maintain
35   concentrations in the blood in the ranges specified

for *in vivo* therapies are contemplated.

As an aid to the administration of effective
amounts of a monoclonal antibody, a diagnostic method
for detecting a monoclonal antibody in the subject's
blood is useful to characterize the fate of the
administered therapeutic composition.

The invention also relates to a method for
preparing a medicament or pharmaceutical composition
comprising the human monoclonal antibodies of the
invention, the medicament being used for immunotherapy
of HIV disease.

D.   Diagnostic Assay Methods

The present invention contemplates various
assay methods for determining the presence, and
preferably amount, of HIV in a sample such as a
biological fluid or tissue sample using a human
monoclonal antibody of this invention as an
immunochemical reagent to form an immunoreaction
product whose amount relates, either directly or
indirectly, to the amount of HIV in the sample.

Those skilled in the art will understand that
there are numerous well known clinical diagnostic
chemistry procedures in which an immunochemical
reagent of this invention can be used to form an
immunoreaction product whose amount relates to the
amount of HIV present in a body sample. Thus, while
exemplary assay methods are described herein, the
invention is not so limited.

Various heterogenous and homogeneous protocols,
either competitive or noncompetitive, can be employed
in performing an assay method of this invention.
Examples of types of immunoassays which can utilize
monoclonal antibodies of the invention are competitive
and non-competitive immunoassays in either a direct or

indirect format. Examples of such immunoassays are
the radioimmunoassay (RIA) and the sandwich
(immunometric) assay. Detection of the antigens using
the monoclonal antibodies of the invention can be done
5   utilizing immunoassays which are run in either the
forward, reverse, or simultaneous modes, including
immunohistochemical assays on physiological samples.
Those of skill in the art will know, or can readily
discern, other immunoassay formats without undue
10   experimentation.

        The monoclonal antibodies of the invention can be
bound to many different carriers and used to detect
the presence of HIV. Examples of well-known carriers
include glass, polystyrene, polypropylene,
15   polyethylene, dextran, nylon, amylases, natural and
modified celluloses, polyacrylamides, agaroses and
magnetite. The nature of the carrier can be either
soluble or insoluble for purposes of the invention.
Those skilled in the art will know of other suitable
20   carriers for binding monoclonal antibodies, or will be
able to ascertain such, using routine experimentation.

        There are many different labels and methods of
labeling known to those of ordinary skill in the art.
Examples of the types of labels which can be used in
25   the present invention include enzymes, radioisotopes,
fluorescent compounds, colloidal metals,
chemiluminescent compounds, and bio-luminescent com-
pounds. Those of ordinary skill in the art will know
of other suitable labels for binding to the monoclonal
30   antibodies of the invention, or will be able to
ascertain such, using routine experimentation.
Furthermore, the binding of these labels to the
monoclonal antibodies of the invention can be done
using standard techniques common to those of ordinary
35   skill in the art.

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For purposes of the invention, HIV may be detected by the monoclonal antibodies of the invention when present in samples of biological fluids and tissues. Any sample containing a detectable amount of HIV can be used. A sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum and the like, or a solid or semi-solid such as tissues, feces, and the like, or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

Another labeling technique which may result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts with avidin, or dinitrophenol, pyridoxal, or fluorescein, which can react with specific anti-hapten antibodies.

The monoclonal antibodies of the invention are suited for use _in vitro_, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier for the detection of HIV in samples, as described above. The monoclonal antibodies in these immunoassays can be detectably labeled in various ways for _in vitro_ use.

In using the human monoclonal antibodies of the invention for the _in vivo_ detection of antigen, the detectably labeled human monoclonal antibody is given in a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled human monoclonal antibody is administered in sufficient quantity to enable detection of the site having the HIV antigen for which the monoclonal antibodies are specific.

The concentration of detectably labeled human monoclonal antibody which is administered should be

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sufficient such that the binding to HIV is detectable
compared to the background. Further, it is desirable
that the detectably labeled monoclonal antibody be
rapidly cleared from the circulatory system in order

5   to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled human
monoclonal antibody for _in vivo_ diagnosis will vary
depending on such factors as age, sex, and extent of
disease of the individual. The dosage of human

10   monoclonal antibody can vary from about 0.01 mg/m^2 to
about 500 mg/m^2, preferably 0.1 mg/m^2 to about 200
mg/m^2, most preferably about 0.1 mg/m^2 to about 10
mg/m^2. Such dosages may vary, for example, depending
on whether multiple injections are given, tissue, and

15   other factors known to those of skill in the art.

For _in vivo_ diagnostic imaging, the type of
detection instrument available is a major factor in
selecting a given radioisotope. The radioisotope
chosen must have a type of decay which is detectable

20   for a given type of instrument. Still another
important factor in selecting a radioisotope for _in
vivo_ diagnosis is that the half-life of the
radioisotope be long enough so that it is still
detectable at the time of maximum uptake by the

25   target, but short enough so that deleterious radiation
with respect to the host is minimized. Ideally, a
radioisotope used for _in vivo_ imaging will lack a
particle emission, but produce a large number of
photons in the 140-250 keV range, which may be readily

30   detected by conventional gamma cameras.

For _in vivo_ diagnosis radioisotopes may be bound
to immunoglobulin either directly or indirectly by
using an intermediate functional group. Intermediate
functional groups which often are used to bind

35   radioisotopes which exist as metallic ions to

immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which

5   can be bound to the monoclonal antibodies of the invention are ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}Tl.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for

10   purposes of _in vivo_ diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are

15   used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

The human monoclonal antibodies of the invention can be used _in vitro_ and _in vivo_ to monitor the course

20   of HIV disease therapy. Thus, for example, by measuring the increase or decrease in the number of cells infected with HIV or changes in the concentration of HIV present in the body or in various body fluids, it would be possible to determine whether

25   a particular therapeutic regimen aimed at ameliorating the HIV disease is effective.

      E.   Diagnostic Systems

The present invention also describes a

30   diagnostic system, preferably in kit form, for assaying for the presence of HIV in a sample according to the diagnostic methods described herein. A diagnostic system includes, in an amount sufficient to perform at least one assay, a subject human monoclonal

35   antibody, as a separately packaged reagent.

36

In another embodiment, a diagnostic system is contemplated for assaying for the presence of an anti-HIV monoclonal antibody in a body fluid sample such as for monitoring the fate of therapeutically

5   administered antibody. The system includes, in an amount sufficient for at least one assay, a subject antibody as a control reagent, and preferably a preselected amount of HIV antigen, each as separately packaged immunochemical reagents.

10   Instructions for use of the packaged reagent are also typically included.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter

15   such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/ sample admixtures, temperature, buffer conditions and the like.

In embodiments for detecting HIV in a body fluid,

20   a diagnostic system of the present invention can include a label or indicating means capable of signaling the formation of an immunocomplex containing a human monoclonal antibody of the present invention.

The word "complex" as used herein refers to the

25   product of a specific binding reaction such as an antibody-antigen reaction. Exemplary complexes are immunoreaction products.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to

30   single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or

35   antibody molecule that is part of an antibody or

monoclonal antibody composition of the present
invention, or used separately, and those atoms or
molecules can be used alone or in conjunction with
additional reagents. Such labels are themselves well-
known in clinical diagnostic chemistry and constitute
a part of this invention only insofar as they are
utilized with otherwise novel proteins methods and/or
systems.

The labeling means can be a fluorescent labeling
agent that chemically binds to antibodies or antigens
without denaturing them to form a fluorochrome (dye)
that is a useful immunofluorescent tracer. Suitable
fluorescent labeling agents are fluorochromes such as
fluorescein isocyanate (FIC), fluorescein
isothiocyanate (FITC), 5-dimethylamine-1-
naphthalenesulfonyl chloride (DANSC),
tetramethylrhodamine isothiocyanate (TRITC),
lissamine, rhodamine 8200 sulphonyl chloride (RB 200
SC) and the like. A description of immunofluorescence
analysis techniques is found in DeLuca,
"Immunofluorescence Analysis", in Antibody As a Tool,
Marchalonis et al., eds., John Wiley & Sons, Ltd., pp.
189-231 (1982), which is incorporated herein by
reference.

In preferred embodiments, the indicating group is
an enzyme, such as horseradish peroxidase (HRP),
glucose oxidase, or the like. In such cases where the
principal indicating group is an enzyme such as HRP or
glucose oxidase, additional reagents are required to
visualize the fact that a receptor-ligand complex
(immunoreactant) has formed. Such additional reagents
for HRP include hydrogen peroxide and an oxidation dye
precursor such as diaminobenzidine. An additional
reagent useful with glucose oxidase is 2,2'-amino-di-
(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling
agents and are used illustratively herein. An
exemplary radiolabeling agent is a radioactive element
that produces gamma ray emissions. Elements which

5   themselves emit gamma rays, such as ^{124}I, ^{125}I, ^{128}I, ^{132}I
and ^{51}Cr represent one class of gamma ray emission-
producing radioactive element indicating groups.
Particularly preferred is ^{125}I. Another group of
useful labeling means are those elements such as ^{11}C,

10   ^{18}F, ^{15}O and ^{13}N which themselves emit positrons. The
positrons so emitted produce gamma rays upon
encounters with electrons present in the animal's
body. Also useful is a beta emitter, such ^{111} indium
of ^3H.

15   The linking of labels, i.e., labeling of,
polypeptides and proteins is well known in the art.
For instance, antibody molecules produced by a
hybridoma can be labeled by metabolic incorporation of
radioisotope-containing amino acids provided as a

20   component in the culture medium. See, for example,
Galfre et al., Meth. Enzymol., 73:3-46 (1981). The
techniques of protein conjugation or coupling through
activated functional groups are particularly
applicable. See, for example, Aurameas et al., Scand.

25   J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et
al., Biotech., 3:889-894 (1984), and U.S. Pat. No.
4,493,795.

The diagnostic systems can also include,
preferably as a separate package, a specific binding

30   agent. A "specific binding agent" is a molecular
entity capable of selectively binding a reagent
species of the present invention or a complex
containing such a species, but is not itself a
polypeptide or antibody molecule composition of the

35   present invention. Exemplary specific binding agents

are second antibody molecules, complement proteins or
fragments thereof, S. aureus protein A, and the like.
Preferably the specific binding agent binds the
reagent species when that species is present as part

5   of a complex.

In preferred embodiments, the specific binding
agent is labeled. However, when the diagnostic system
includes a specific binding agent that is not labeled,
the agent is typically used as an amplifying means or

10   reagent. In these embodiments, the labeled specific
binding agent is capable of specifically binding the
amplifying means when the amplifying means is bound to
a reagent species-containing complex.

The diagnostic kits of the present invention can

15   be used in an "ELISA" format to detect the quantity of
an APC inhibitor of this invention in a vascular fluid
sample such as blood, serum, or plasma. "ELISA"
refers to an enzyme-linked immunosorbent assay that
employs an antibody or antigen bound to a solid phase

20   and an enzyme-antigen or enzyme-antibody conjugate to
detect and quantify the amount of an antigen present
in a sample. A description of the ELISA technique is
found in Chapter 22 of the 4th Edition of Basic and
Clinical Immunology by D.P. Sites et al., published by

25   Lange Medical Publications of Los Altos, CA in 1982
and in U.S. Patents No. 3,654,090; No. 3,850,752; and
No. 4,016,043, which are all incorporated herein by
reference.

Thus, in some embodiments, a human monoclonal

30   antibody of the present invention can be affixed to a
solid matrix to form a solid support that comprises a
package in the subject diagnostic systems.

A reagent is typically affixed to a solid matrix
by adsorption from an aqueous medium although other

35   modes of affixation applicable to proteins and

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polypeptides well known to those skilled in the art, can be used.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron to about 5 millimeters in diameter available from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

The term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene and polycarbonate), paper, foil and the like capable of holding within fixed limits a diagnostic reagent such as a monoclonal antibody of the present invention. Thus, for example, a package can be a bottle, vial, plastic and plastic-foil

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laminated envelope or the like container used to
contain a contemplated diagnostic reagent or it can be
a microtiter plate well to which microgram quantities
of a contemplated diagnostic reagent have been
5   operatively affixed, i.e., linked so as to be capable
of being immunologically bound by an antibody or
polypeptide to be detected.

The materials for use in the assay of the
invention are ideally suited for the preparation of a
10   kit. Such a kit may comprise a carrier means being
compartmentalized to receive in close confinement one
or more container means such as vials, tubes, and the
like, each of the container means comprising one of
the separate elements to be used in the method. For
15   example, one of the container means may comprise a
human monoclonal antibody of the invention which is,
or can be, detectably labelled. The kit may also have
containers containing any of the other above-recited
immunochemical reagents used to practice the
20   diagnostic methods.

        F.   Methods for Producing an HIV-Neutralizing
              Human Monoclonal Antibody

The present invention describes methods for
25   producing novel HIV-neutralizing human monoclonal
antibodies. The methods are based generally on the
use of combinatorial libraries of antibody molecules
which can be produced from a variety of sources, and
include naive libraries, modified libraries, and
30   libraries produced directly from human donors
exhibiting an HIV-specific immune response.

The combinatorial library production and
manipulation methods have been extensively described
in the literature, and will not be reviewed in detail
35   herein, except for those feature required to make and

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use unique embodiments of the present invention.
However, the methods generally involve the use of a
filamentous phage (phagemid) surface expression vector
system for cloning and expressing antibody species of
5   the library. Various phagemid cloning systems to
produce combinatorial libraries have been described by
others. See, for example the preparation of
combinatorial antibody libraries on phagemids as
described by Kang et al., Proc. Natl. Acad. Sci., USA,
10   88:4363-4366 (1991); Barbas et al., Proc. Natl. Acad.
Sci., USA, 88:7978-7982 (1991); Zebedee et al., Proc.
Natl. Acad. Sci., USA, 89:3175-3179 (1992); Kang et
al., Proc. Natl. Acad. Sci., USA, 88:11120-11123
(1991); Barbas et al., Proc. Natl. Acad. Sci., USA,
15   89:4457-4461 (1992); and Gram et al., Proc. Natl.
Acad. Sci., USA, 89:3576-3580 (1992), which references
are hereby incorporated by reference.

In one embodiment, the method involves preparing
a phagemid library of human monoclonal antibodies by
20   using donor immune cell messenger RNA from HIV-
infected donors. The donors can be symptomatic of
AIDS, but in preferred embodiments the donor is
asymptomatic, as the resulting library contains a
substantially higher number of HIV-neutralizing human
25   monoclonal antibodies.

In another embodiment, the donor is naive
relative to an immune response to HIV, i.e., the donor
is not HIV-infected. Alternatively, the library can
be synthetic, or can be derived from a donor who has
30   an immune response to other antigens.

The method for producing a human monoclonal
antibody generally involves (1) preparing separate H
and L chain-encoding gene libraries in cloning vectors
using human immunoglobulin genes as a source for the
35   libraries, (2) combining the H and L chain encoding

gene libraries into a single dicistronic expression
vector capable of expressing and assembling a
heterodimeric antibody molecule, (3) expressing the
assembled heterodimeric antibody molecule on the

5   surface of a filamentous phage particle, (4) isolating
the surface-expressed phage particle using
immunoaffinity techniques such as panning of phage
particles against a preselected antigen, thereby
isolating one or more species of phagemid containing

10   particular H and L chain-encoding genes and antibody
molecules that immunoreact with the preselected
antigen.

        As described herein the Examples, the resulting
phagemid library can be manipulated to increase and/or

15   alter the immunospecificities of the monoclonal
antibodies of the library to produce and subsequently
identify additional, desirable, human monoclonal
antibodies of the present invention.

        For example, the heavy (H) chain and light (L)

20   chain immunoglobulin molecule encoding genes can be
randomly mixed (shuffled) to create new HL pairs in an
assembled immunoglobulin molecule. Additionally,
either or both the H and L chain encoding genes can be
mutagenized in the complementarity determining region

25   (CDR) of the variable region of the immunoglobulin
polypeptide, and subsequently screened for desirable
immunoreaction and neutralization capabilities.

        In one embodiment, the H and L genes can be
cloned into separate, monocistronic expression

30   vectors, referred to as a "binary" system described
further herein. In this method, step (2) above
differs in that the combining of H and L chain
encoding genes occurs by the co-introduction of the
two binary plasmids into a single host cell for

35   expression and assembly of a phagemid having the

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surface accessible antibody heterodimer molecule.

In one shuffling embodiment, the shuffling can be accomplished with the binary expression vectors, each capable of expressing a single heavy or light chain
5   encoding gene, as described in Example 6.

In the present methods, the antibody molecules are monoclonal because the cloning methods allow for the preparation of clonally pure species of antibody producing cell lines. In addition, the monoclonal
10   antibodies are human because the H and L chain encoding genes are derived from human immunoglobulin producing immune cells, such as spleen, thymus, bone marrow, and the like.

The method of producing a HIV-neutralizing human
15   monoclonal antibody also requires that the resulting antibody library, immunoreactive with a preselected HIV antigen, is screened for the presence of antibody species which have the capacity to neutralize HIV in one or more of the assays described herein for
20   determining neutralization capacity. Thus, a preferred library of antibody molecules is first produced which binds to an HIV antigen, preferably gp160, gp120, gp41, the V3 loop region of gp160, or the CD4 binding site of gp120 and gp41, and then is
25   screened for the presence of HIV-neutralizing antibodies as described herein.

Additional libraries can be screened from shuffled libraries for additional HIV-immunoreactive and neutralizing human monoclonal antibodies.
30   As a further characterization of the present invention the nucleotide and corresponding amino acid residue sequence of the antibody molecule's H or L chain encoding gene is determined by nucleic acid sequencing. The primary amino acid residue sequence
35   information provides essential information regarding

the antibody molecule's epitope reactivity.

Sequence comparisons of identified HIV-immunoreactive monoclonal antibody variable chain region sequences are shown herein in Figures 10-13. The sequences are aligned based on sequence homology, and groups of related antibody molecules are identified thereby in which heavy chain or light chain genes share substantial sequence homology.

An exemplary preparation of a human monoclonal antibody is described in the Examples. The isolation of a particular vector capable of expressing an antibody of interest involves the introduction of the dicistronic expression vector into a host cell permissive for expression of filamentous phage genes and the assembly of phage particles. Where the binary vector system is used, both vectors are introduced in the host cell. Typically, the host is E. coli. Thereafter, a helper phage genome is introduced into the host cell containing the immunoglobulin expression vector(s) to provide the genetic complementation necessary to allow phage particles to be assembled. The resulting host cell is cultured to allow the introduced phage genes and immunoglobulin genes to be expressed, and for phage particles to be assembled and shed from the host cell. The shed phage particles are then harvested (collected) from the host cell culture media and screened for desirable immunoreaction and neutralization properties. Typically, the harvested particles are "panned" for immunoreaction with a preselected antigen. The strongly immunoreactive particles are then collected, and individual species of particles are clonally isolated and further screened for HIV neutralization. Phage which produce neutralizing antibodies are selected and used as a source of a human HIV neutralizing monoclonal antibody

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of this invention.

Human monoclonal antibodies of this invention can
also be produced by altering the nucleotide sequence
of a polynucleotide sequence that encodes a heavy or
5   light chain of a monoclonal antibody of this
invention. For example, by site directed mutagenesis,
one can alter the nucleotide sequence of an expression
vector and thereby introduce changes in the resulting
expressed amino acid residue sequence. Thus one can
10   take the polynucleotide of SEQ ID NO 66, for example,
and convert it into the polynucleotide of SEQ ID NO
67. Similarly, one can take a known polynucleotide
and randomly alter it by random mutagenesis,
reintroduce the altered polynucleotide into an
15   expression system and subsequently screen the product
H:L pair for HIV-neutralizing activity.

Site-directed and random mutagenesis methods are
well known in the polynucleotide arts, and are not to
be construed as limiting as methods for altering the
20   nucleotide sequence of a subject polynucleotide.

Due to the presence of the phage particle in an
immunoaffinity isolated antibody, one embodiment
involves the manipulation of the resulting cloned
genes to truncate the immunoglobulin-coding gene such
25   that a soluble Fab fragment is secreted by the host E.
coli cell containing the phagemid vector. Thus, the
resulting manipulated cloned immunoglobulin genes
produce a soluble Fab which can be readily
characterized in ELISA assays for epitope binding
30   studies, in competition assays with known anti-HIV
antibody molecules, and in HIV neutralization assays.
The solubilized Fab provides a reproducible and
comparable antibody preparation for comparative and
characterization studies.

35   The preparation of soluble Fab is generally

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described in the immunological arts, and can be
conducted as described herein in Example 2b6), or as
described by Burton et al., Proc. Natl. Acad. Sci.,
USA, 88:10134-10137 (1991).

5

     G.   Expression Vectors and Polynucleotides for
            Expressing Anti-HIV Monoclonal Antibodies
           The preparation of human monoclonal
antibodies of this invention depends, in one
embodiment, on the cloning and expression vectors used

10

to prepare the combinatorial antibody libraries
described herein. The cloned immunoglobulin heavy and
light chain genes can be shuttled between lambda
vectors, phagemid vectors and plasmid vectors at

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various stages of the methods described herein.
           The phagemid vectors produce fusion proteins that
are expressed on the surface of an assembled
filamentous phage particle.
           A preferred phagemid vector of the present

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invention is a recombinant DNA (rDNA) molecule
containing a nucleotide sequence that codes for and is
capable of expressing a fusion polypeptide containing,
in the direction of amino- to carboxy-terminus, (1) a
prokaryotic secretion signal domain, (2) a

25

heterologous polypeptide defining an immunoglobulin
heavy or light chain variable region, and (3) a
filamentous phage membrane anchor domain. The vector
includes DNA expression control sequences for
expressing the fusion polypeptide, preferably

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prokaryotic control sequences.
           The filamentous phage membrane anchor is
preferably a domain of the cpIII or cpVIII coat
protein capable of associating with the matrix of a
filamentous phage particle, thereby incorporating the

35

fusion polypeptide onto the phage surface.

The secretion signal is a leader peptide domain
of a protein that targets the protein to the
periplasmic membrane of gram negative bacteria. A
preferred secretion signal is a pelB secretion signal.
5   The predicted amino acid residue sequences of the
secretion signal domain from two pelB gene product
variants from Erwinia carotova are described in Lei et
al., Nature, 331:543-546 (1988).

The leader sequence of the pelB protein has
10   previously been used as a secretion signal for fusion
proteins (Better et al., Science, 240:1041-1043
(1988); Sastry et al., Proc. Natl. Acad. Sci., USA,
86:5728-5732 (1989); and Mullinax et al., Proc. Natl.
Acad. Sci., USA, 87:8095-8099 (1990)). Amino acid
15   residue sequences for other secretion signal
polypeptide domains from E. coli useful in this
invention as described in Oliver, Escherichia coli
and Salmonella Typhimurium, Neidhard, F.C. (ed.),
American Society for Microbiology, Washington, D.C.,
20   1:56-69 (1987).

Preferred membrane anchors for the vector are
obtainable from filamentous phage M13, f1, fd, and
equivalent filamentous phage. Preferred membrane
anchor domains are found in the coat proteins encoded
25   by gene III and gene VIII. The membrane anchor domain
of a filamentous phage coat protein is a portion of
the carboxy terminal region of the coat protein and
includes a region of hydrophobic amino acid residues
for spanning a lipid bilayer membrane, and a region of
30   charged amino acid residues normally found at the
cytoplasmic face of the membrane and extending away
from the membrane.

In the phage f1, gene VIII coat protein's
membrane spanning region comprises residue Trp-26
35   through Lys-40, and the cytoplasmic region comprises

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the carboxy-terminal 11 residues from 41 to 52 (Ohkawa
et al., J. Biol. Chem., 256:9951-9958 (1981)). An
exemplary membrane anchor would consist of residues 26
to 40 of cpVIII. Thus, the amino acid residue
5   sequence of a preferred membrane anchor domain is
derived from the M13 filamentous phage gene VIII coat
protein (also designated cpVIII or CP 8). Gene VIII
coat protein is present on a mature filamentous phage
over the majority of the phage particle with typically
10   about 2500 to 3000 copies of the coat protein.

In addition, the amino acid residue sequence of
another preferred membrane anchor domain is derived
from the M13 filamentous phage gene III coat protein
(also designated cpIII). Gene III coat protein is
15   present on a mature filamentous phage at one end of
the phage particle with typically about 4 to 6 copies
of the coat protein.

For detailed descriptions of the structure of
filamentous phage particles, their coat proteins and
20   particle assembly, see the reviews by Rached et al.,
Microbiol. Rev., 50:401-427 (1986); and Model et al.,
in "The Bacteriophages: Vol. 2", R. Calendar, ed.
Plenum Publishing Co., pp. 375-456 (1988).

DNA expression control sequences comprise a set
25   of DNA expression signals for expressing a structural
gene product and include both 5' and 3' elements, as
is well known, operatively linked to the cistron such
that the cistron is able to express a structural gene
product. The 5' control sequences define a promoter
30   for initiating transcription and a ribosome binding
site operatively linked at the 5' terminus of the
upstream translatable DNA sequence.

To achieve high levels of gene expression in E.
coli, it is necessary to use not only strong promoters
35   to generate large quantities of mRNA, but also

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ribosome binding sites to ensure that the mRNA is
efficiently translated. In _E. coli_, the ribosome
binding site includes an initiation codon (AUG) and a
sequence 3-9 nucleotides long located 3-11 nucleotides
5   upstream from the initiation codon (Shine et al.,
Nature, 254:34 (1975). The sequence, AGGAGGU, which
is called the Shine-Dalgarno (SD) sequence, is
complementary to the 3' end of _E. coli_ 16S rRNA.
Binding of the ribosome to mRNA and the sequence at
10   the 3' end of the mRNA can be affected by several
factors:

            (i)   The degree of complementarity between
the SD sequence and 3' end of the 16S rRNA.

            (ii) The spacing and possibly the DNA
15   sequence lying between the SD sequence and the AUG.
Roberts et al., _Proc. Natl. Acad. Sci., USA_, 76:760,
(1979a); Roberts et al., _Proc. Natl. Acad. Sci. USA_,
76:5596 (1979b); Guarente et al., _Science_, 209:1428
(1980); and Guarente et al., _Cell_, 20:543 (1980).
20   Optimization is achieved by measuring the level of
expression of genes in plasmids in which this spacing
is systematically altered. Comparison of different
mRNAs shows that there are statistically preferred
sequences from positions -20 to +13 (where the A of
25   the AUG is position 0). Gold et al., _Annu. Rev.
Microbiol._, 35:365 (1981). Leader sequences have been
shown to influence translation dramatically. Roberts
et al., 1979 a, b _supra_.

            (iii) The nucleotide sequence following the
30   AUG, which affects ribosome binding. Taniguchi et
al., _J. Mol. Biol._, 118:533 (1978).

       The 3' control sequences define at least one
termination (stop) codon in frame with and operatively
linked to the heterologous fusion polypeptide.
35   In preferred embodiments, the vector utilized

includes a prokaryotic origin of replication or
replicon, i.e., a DNA sequence having the ability to
direct autonomous replication and maintenance of the
recombinant DNA molecule extra chromosomally in a
5   prokaryotic host cell, such as a bacterial host cell,
transformed therewith. Such origins of replication
are well known in the art. Preferred origins of
replication are those that are efficient in the host
organism. A preferred host cell is E. coli. For use
10   of a vector in E. coli, a preferred origin of
replication is ColE1 found in pBR322 and a variety of
other common plasmids. Also preferred is the p15A
origin of replication found on pACYC and its
derivatives. The ColE1 and p15A replicon have been
15   extensively utilized in molecular biology, are
available on a variety of plasmids and are described
at least by Sambrook et al., in "Molecular Cloning: a
Laboratory Manual", 2nd edition, Cold Spring Harbor
Laboratory Press (1989).
20   The ColE1 and p15A replicons are particularly
preferred for use in one embodiment of the present
invention where two "binary" plasmids are utilized
because they each have the ability to direct the
replication of plasmid in E. coli while the other
25   replicon is present in a second plasmid in the same E.
coli cell. In other words, ColE1 and p15A are non-
interfering replicons that allow the maintenance of
two plasmids in the same host (see, for example,
Sambrook et al., supra, at pages 1.3-1.4). This
30   feature is particularly important in the binary
vectors embodiment of the present invention because a
single host cell permissive for phage replication must
support the independent and simultaneous replication
of two separate vectors, namely a first vector for
35   expressing a heavy chain polypeptide, and a second

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vector for expressing a light chain polypeptide.

In addition, those embodiments that include a
prokaryotic replicon can also include a gene whose
expression confers a selective advantage, such as drug
5   resistance, to a bacterial host transformed therewith.
Typical bacterial drug resistance genes are those that
confer resistance to ampicillin, tetracycline,
neomycin/kanamycin or cholamphenicol. Vectors
typically also contain convenient restriction sites
10   for insertion of translatable DNA sequences.
Exemplary vectors are the plasmids pUC8, pUC9, pBR322,
and pBR329 available from BioRad Laboratories,
(Richmond, CA) and pPL and pKK223 available from
Pharmacia, (Piscataway, NJ).

15   A vector for expression of a monoclonal antibody
of the invention on the surface of a filamentous phage
particle is a recombinant DNA (rDNA) molecule adapted
for receiving and expressing translatable first and
second DNA sequences in the form of first and second
20   polypeptides wherein one of the polypeptides is fused
to a filamentous phage coat protein membrane anchor.
That is, at least one of the polypeptides is a fusion
polypeptide containing a filamentous phage membrane
anchor domain, a prokaryotic secretion signal domain,
25   and an immunoglobulin heavy or light chain variable
domain.

A DNA expression vector for expressing a
heterodimeric antibody molecule provides a system for
independently cloning (inserting) the two translatable
30   DNA sequences into two separate cassettes present in
the vector, to form two separate cistrons for
expressing the first and second polypeptides of the
antibody molecule, or the ligand binding portions of
the polypeptides that comprise the antibody molecule
35   (i.e., the H and L variable regions of an

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immunoglobulin molecule). The DNA expression vector
for expressing two cistrons is referred to as a
dicistronic expression vector.

The vector comprises a first cassette that
includes upstream and downstream translatable DNA
sequences operatively linked via a sequence of
nucleotides adapted for directional ligation to an
insert DNA. The upstream translatable sequence
encodes the secretion signal as defined herein. The
downstream translatable sequence encodes the
filamentous phage membrane anchor as defined herein.
The cassette preferably includes DNA expression
control sequences for expressing the receptor
polypeptide that is produced when an insert
translatable DNA sequence (insert DNA) is
directionally inserted into the cassette via the
sequence of nucleotides adapted for directional
ligation. The filamentous phage membrane anchor is
preferably a domain of the cpIII or cpVIII coat
protein capable of binding the matrix of a filamentous
phage particle, thereby incorporating the fusion
polypeptide onto the phage surface.

The receptor expressing vector also contains a
second cassette for expressing a second receptor
polypeptide. The second cassette includes a second
translatable DNA sequence that encodes a secretion
signal, as defined herein, operatively linked at its
3' terminus via a sequence of nucleotides adapted for
directional ligation to a downstream DNA sequence of
the vector that typically defines at least one stop
codon in the reading frame of the cassette. The
second translatable DNA sequence is operatively linked
at its 5' terminus to DNA expression control sequences
forming the 5' elements. The second cassette is
capable, upon insertion of a translatable DNA sequence

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(insert DNA), of expressing the second fusion
polypeptide comprising a receptor of the secretion
signal with a polypeptide coded by the insert DNA.

An upstream translatable DNA sequence encodes a
5   prokaryotic secretion signal as described earlier.
The upstream translatable DNA sequence encoding the
pelB secretion signal is a preferred DNA sequence for
inclusion in a receptor expression vector. A
downstream translatable DNA sequence encodes a
10   filamentous phage membrane anchor as described
earlier. Thus, a downstream translatable DNA sequence
encodes an amino acid residue sequence that
corresponds, and preferably is identical, to the
membrane anchor domain of either a filamentous phage
15   gene III or gene VIII coat polypeptide.

A cassette in a DNA expression vector of this
invention is the region of the vector that forms, upon
insertion of a translatable DNA sequence (insert DNA),
a sequence of nucleotides capable of expressing, in an
20   appropriate host, a fusion polypeptide. The
expression-competent sequence of nucleotides is
referred to as a cistron. Thus, the cassette
comprises DNA expression control elements operatively
linked to the upstream and downstream translatable DNA
25   sequences. A cistron is formed when a translatable
DNA sequence is directionally inserted (directionally
ligated) between the upstream and downstream sequences
via the sequence of nucleotides adapted for that
purpose. The resulting three translatable DNA
30   sequences, namely the upstream, the inserted and the
downstream sequences, are all operatively linked in
the same reading frame.

Thus, a DNA expression vector for expressing an
antibody molecule provides a system for cloning
35   translatable DNA sequences into the cassette portions

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of the vector to produce cistrons capable of
expressing the first and second polypeptides, i.e.,
the heavy and light chains of a monoclonal antibody.

As used herein, the term "vector" refers to a
nucleic acid molecule capable of transporting between
different genetic environments another nucleic acid to
which it has been operatively linked. Preferred
vectors are those capable of autonomous replication
and expression of structural gene products present in
the DNA segments to which they are operatively linked.
Vectors, therefore, preferably contain the replicons
and selectable markers described earlier.

As used herein with regard to DNA sequences or
segments, the phrase "operatively linked" means the
sequences or segments have been covalently joined,
preferably by conventional phosphodiester bonds, into
one strand of DNA, whether in single or double
stranded form. The choice of vector to which
transcription unit or a cassette of this invention is
operatively linked depends directly, as is well known
in the art, on the functional properties desired,
e.g., vector replication and protein expression, and
the host cell to be transformed, these being
limitations inherent in the art of constructing
recombinant DNA molecules.

A sequence of nucleotides adapted for directional
ligation, i.e., a polylinker, is a region of the DNA
expression vector that (1) operatively links for
replication and transport the upstream and downstream
translatable DNA sequences and (2) provides a site or
means for directional ligation of a DNA sequence into
the vector. Typically, a directional polylinker is a
sequence of nucleotides that defines two or more
restriction endonuclease recognition sequences, or
restriction sites. Upon restriction cleavage, the two

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sites yield cohesive termini to which a translatable
DNA sequence can be ligated to the DNA expression
vector. Preferably, the two restriction sites
provide, upon restriction cleavage, cohesive termini
5   that are non-complementary and thereby permit
directional insertion of a translatable DNA sequence
into the cassette. In one embodiment, the directional
ligation means is provided by nucleotides present in
the upstream translatable DNA sequence, downstream
10   translatable DNA sequence, or both. In another
embodiment, the sequence of nucleotides adapted for
directional ligation comprises a sequence of
nucleotides that defines multiple directional cloning
means. Where the sequence of nucleotides adapted for
15   directional ligation defines numerous restriction
sites, it is referred to as a multiple cloning site.

In a preferred embodiment, a DNA expression
vector is designed for convenient manipulation in the
form of a filamentous phage particle encapsulating a
20   genome according to the teachings of the present
invention. In this embodiment, a DNA expression
vector further contains a nucleotide sequence that
defines a filamentous phage origin of replication such
that the vector, upon presentation of the appropriate
25   genetic complementation, can replicate as a
filamentous phage in single stranded replicative form
and be packaged into filamentous phage particles.
This feature provides the ability of the DNA
expression vector to be packaged into phage particles
30   for subsequent segregation of the particle, and vector
contained therein, away from other particles that
comprise a population of phage particles.

A filamentous phage origin of replication is a
region of the phage genome, as is well known, that
35   defines sites for initiation of replication,

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termination of replication and packaging of the
replicative form produced by replication (see, for
example, Rasched et al., _Microbiol. Rev._, 50:401-427
(1986); and Horiuchi, _J. Mol. Biol._, 188:215-223

5   (1986)).

A preferred filamentous phage origin of
replication for use in the present invention is an
M13, f1 or fd phage origin of replication (Short et
al., _Nucl. Acids Res._, 16:7583-7600 (1988)).

10   Preferred DNA expression vectors for cloning and
expression a human monoclonal antibody of this
invention are the dicistronic expression vectors
pComb8, pComb2-8, pComb3, pComb2-3 and pComb2-3',
described herein.

15   A particularly preferred vector of the present
invention includes a polynucleotide sequence that
encodes a heavy or light chain variable region of a
human monoclonal antibody of the present invention.
Particularly preferred are vectors that include a

20   nucleotide sequence that encodes a heavy or light
chain amino acid residue sequence shown in Figures 10-
13, that encodes a heavy or light chain having the
binding specificity of those sequences shown in
Figures 10-13, or that encodes a heavy or light chain

25   having conservative substitutions relative to a
sequence shown in Figures 10-13, and complementary
polynucleotide sequences thereto.

Insofar as polynucleotides are component parts of
a DNA expression vector for producing a human

30   monoclonal antibody heavy or light chain
immunoglobulin variable region amino acid residue
sequence, the invention also contemplates isolated
polynucleotides that encode such heavy or light chain
sequences.

35   It is to be understood that, due to the genetic

code and its attendant redundancies, numerous
polynucleotide sequences can be designed that encode a
contemplated heavy or light chain immunoglobulin
variable region amino acid residue sequence. Thus,
the invention contemplates such alternate
polynucleotide sequences incorporating the features of
the redundancy of the genetic code.

Insofar as the expression vector for producing a
human monoclonal antibody of this invention is carried
in a host cell compatible with expression of the
antibody, the invention contemplates a host cell
containing a vector or polynucleotide of this
invention. A preferred host cell is E. coli, as
described herein.

E. coli cultures containing preferred expression
vectors that produce a human monoclonal antibody of
this invention were deposited pursuant to Budapest
Treaty requirements with the American Type Culture
Collection (ATCC), Rockville, MD, as described herein.

Examples

The following examples are intended to
illustrate, but not limit, the scope of the invention.

1.   Construction of a Dicistronic Expression Vector
     for Producing a Heterodimeric Receptor on Phage
     Particles

To obtain a vector system for generating a large
number of Fab antibody fragments that can be screened
directly, expression libraries in bacteriophage Lambda
have previously been constructed as described in Huse
et al., Science, 246:1275-1281 (1989). These systems
did not contain design features that provide for the
expressed Fab to be targeted to the surface of a
filamentous phage particle.

The main criterion used in choosing a vector
system was the necessity of generating the largest
number of Fab fragments which could be screened
directly. Bacteriophage Lambda was selected as the
starting point to develop an expression vector for
three reasons. First, in vitro packaging of phage DNA
was the most efficient method of reintroducing DNA
into host cells. Second, it was possible to detect
protein expression at the level of single phage
plaques. Finally, the screening of phage libraries
typically involved less difficulty with nonspecific
binding. The alternative, plasmid cloning vectors,
are only advantageous in the analysis of clones after
they have been identified. This advantage was not
lost in the present system because of the use of a
dicistronic expression vector such as pCombVIII,
thereby permitting a plasmid containing the heavy
chain, light chain, or Fab expressing inserts to be
excised.

a.   Construction of Dicistronic Expression
Vector pCOMB

1)   Preparation of Lambda Zap ^{TM}II

Lambda Zap^{TM} II is a derivative of the
original Lambda Zap (ATCC Accession No. 40,298) that
maintains all of the characteristics of the original
Lambda Zap including 6 unique cloning sites, fusion
protein expression, and the ability to rapidly excise
the insert in the form of a phagemid (Bluescript SK-),
but lacks the SAM 100 mutation, allowing growth on
many Non-Sup F strains, including XL1-Blue. The
Lambda Zap^{TM} II was constructed as described in Short
et al., Nuc. Acids Res., 16:7583-7600, 1988, by
replacing the Lambda S gene contained in a 4254 base
pair (bp) DNA fragment produced by digesting Lambda
Zap with the restriction enzyme Nco I. This 4254 bp

DNA fragment was replaced with the 4254 bp DNA
fragment containing the Lambda S gene isolated from
Lambda gt10 (ATCC # 40,179) after digesting the vector
with the restriction enzyme Nco I. The 4254 bp DNA
5   fragment isolated from lambda gt10 was ligated into
the original Lambda Zap vector using T4 DNA ligase and
standard protocols such as those described in Current
Protocols in Molecular Biology, Ausubel et al., eds.,
John Wiley and Sons, NY, 1987, to form Lambda Zap™
10   II.

2)   Preparation of Lambda Hc2

To express a plurality of V_H-coding DNA
homologs in an E. coli host cell, a vector designated
Lambda Hc2 was constructed. The vector provided the
15   following: the capacity to place the V_H-coding DNA
homologs in the proper reading frame; a ribosome
binding site as described by Shine et al., Nature,
254:34 (1975); a leader sequence directing the
expressed protein to the periplasmic space designated
20   the pelB secretion signal; a polynucleotide sequence
that coded for a known epitope (epitope tag); and also
a polynucleotide that coded for a spacer protein
between the V_H-coding DNA homolog and the
polynucleotide coding for the epitope tag. Lambda Hc2
25   has been previously described by Huse et al., Science,
246:1275-1281 (1989).

To prepare Lambda Hc2, a synthetic DNA sequence
containing all of the above features was constructed
by designing single stranded polynucleotide segments
30   of 20-40 bases that would hybridize to each other and
form the double stranded synthetic DNA sequence shown
in Figure 1. The individual single-stranded
polynucleotide segments are shown in Table 1.

Polynucleotides N2, N3, N9-4, N11, N10-5, N6, N7
35   and N8 (Table 1) were kinased by adding 1 µl of each

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polynucleotide 0.1 micrograms/microliter (μg/μl) and
20 units of T_4 polynucleotide kinase to a solution
containing 70 mM Tris-HCl (Tris[hydroxymethyl]
aminomethane hydrochloride) at pH 7.6, 10 mM $MgCl_2$, 5
5   mM dithiothreitol (DTT), 10 mM beta-mercaptoethanol,
500 micrograms per milliliter (μg/ml) bovine serum
albumin (BSA). The solution was maintained at 37
degrees Centigrade (37°C) for 30 minutes and the
reaction stopped by maintaining the solution at 65°C
10   for 10 minutes. The two end polynucleotides, 20
nanograms (ng) of polynucleotides N1 and
polynucleotides N12, were added to the above kinasing
reaction solution together with 1/10 volume of a
solution containing 20 mM Tris-HCl at pH 7.4, 2.0 mM
15     $MgCl_2$ and 50 mM NaCl. This solution was heated to
70°C for 5 minutes and allowed to cool to room
temperature, approximately 25°C, over 1.5 hours in a
500 ml beaker of water. During this time period all
10 polynucleotides annealed to form the double
20   stranded synthetic DNA insert shown in Figure 1. The
individual polynucleotides were covalently linked to
each other to stabilize the synthetic DNA insert by
adding 40 μl of the above reaction to a solution
containing 50 mM Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM
25   DTT, 1 mM adenosine triphosphate (ATP) and 10 units of
T4 DNA ligase. This solution was maintained at 37°C
for 30 minutes and then the T4 DNA ligase was
inactivated by maintaining the solution at 65°C for 10
minutes. The end polynucleotides were kinased by
30   mixing 52 μl of the above reaction, 4 μl of a solution
containing 10 mM ATP and 5 units of T4 polynucleotide
kinase. This solution was maintained at 37°C for 30
minutes and then the T4 polynucleotide kinase was
inactivated by maintaining the solution at 65°C for 10
35   minutes.

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Table 1

SEQ
ID NO

(15)	N1)	5' GGCCGCAAATTCTATTTCAAGGAGACAGTCAT 3'
(16)	N2)	5' AATGAAATACCTATTGCCTACGGCAGCCGCTGGATT 3'
(17)	N3)	5' GTTATTACTCGCTGCCCAACCAGCCATGGCCC 3'
(18)	N6)	5' CAGTTTCACCTGGGCCATGGCTGGTTGGG 3'
(19)	N7)	5' CAGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAG 3'
(20)	N8)	5' GTATTTCATTATGACTGTCTCCTTGAAATAGAATTTGC 3'
(21)	N9-4)	5' AGGTGAAACTGCTCGAGATTTCTAGACTAGTTACCCGTAC 3'
(22)	N10-5)	5' CGGAACGTCGTACGGGTAACTAGTCTAGAAATCTCGAG 3'
(23)	N11)	5' GACGTTCCGGACTACGGTTCTTAATAGAATTCG 3'
(24)	N12)	5' TCGACGAATTCTATTAAGAACCGTAGTC 3'

The completed synthetic DNA insert was ligated
directly into the Lambda Zap™ II vector described in
Example 1a1) that had been previously digested with
the restriction enzymes, Not I and Xho I. The
ligation mixture was packaged according to the
manufacture's instructions using Gigapack II Gold
packing extract available from Stratagene, La Jolla,
California. The packaged ligation mixture was plated
on XL1-Blue cells (Stratagene). Individual lambda
plaques were cored and the inserts excised according
to the in vivo excision protocol for Lambda Zap™ II
provided by the manufacturer (Stratagene). This in
vivo excision protocol moved the cloned insert from
the Lambda Hc2 vector into a phagemid vector to allow
easy for manipulation and sequencing. The accuracy of
the above cloning steps was confirmed by sequencing
the insert using the Sanger dideoxy method described
in by Sanger et al., Proc. Natl. Acad. Sci., USA,
74:5463-5467 (1977) and using the manufacture's
instructions in the AMV Reverse Transcriptase 35S-ATP

sequencing kit (Stratagene). The sequence of the
resulting double-stranded synthetic DNA insert in the
V_H expression vector (Lambda Hc2) is shown in Figure
1. The sequence of each strand (top and bottom) of

5   Lambda Hc2 is listed in the Sequence Listing as SEQ ID
NO 1 and SEQ ID NO 2, respectively. The resultant
Lambda Hc2 expression vector is shown in Figure 2.

3)   Preparation of Lambda Lc2

10   To express a plurality of V_L-coding DNA
homologs in an E. coli host cell, a vector designated
Lambda Lc2 was constructed having the capacity to
place the V_L-coding DNA homologs in the proper reading
frame, provided a ribosome binding site as described

15   by Shine et al., Nature, 254:34 (1975), provided the
pelB gene leader sequence secretion signal that has
been previously used to successfully secrete Fab
fragments in E. coli by Lei et al., J. Bac., 169:4379
(1987) and Better et al., Science, 240:1041 (1988),

20   and also provided a polynucleotide containing a
restriction endonuclease site for cloning. Lambda Lc2
has been previously described by Huse et al., Science,
246:1275-1281 (1989).
         A synthetic DNA sequence containing all of the

25   above features was constructed by designing single
stranded polynucleotide segments of 20-60 bases that
would hybridize to each other and form the double
stranded synthetic DNA sequence shown in Figure 3.
The sequence of each individual single-stranded

30   polynucleotide segment (O1-O8) within the double
stranded synthetic DNA sequence is shown in Table 2.
         Polynucleotides O2, O3, O4, O5, O6 and O7 (Table
2) were kinased by adding 1 μl (0.1 μg/μl) of each
polynucleotide and 20 units of T_4 polynucleotide

35   kinase to a solution containing 70 mM Tris-HCl at pH

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7.6, 10 mM MgCl$_2$, 5 mM DTT, 10 mM beta-mercaptoethanol, 500 μg/ml of BSA. The solution was maintained at 37°C for 30 minutes and the reaction stopped by maintaining the solution at 65°C for 10

5   minutes. The 20 ng each of the two end polynucleotides, 01 and 08, were added to the above kinasing reaction solution together with 1/10 volume of a solution containing 20.0 mM Tris-HCl at pH 7.4, 2.0 mM MgCl$_2$ and 15.0 mM sodium chloride (NaCl). This

10   solution was heated to 70°C for 5 minutes and allowed to cool to room temperature, approximately 25°C, over 1.5 hours in a 500 ml beaker of water. During this time period all 8 polynucleotides annealed to form the double stranded synthetic DNA insert shown in Figure

15   3. The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert by adding 40 μl of the above reaction to a solution containing 50 mM Tris-HCl at pH 7.5, 7 mM MgCl$_2$, 1 mM DTT, 1 mM ATP and 10 units of T4 DNA

20   ligase. This solution was maintained at 37°C for 30 minutes and then the T4 DNA ligase was inactivated by maintaining the solution at 65°C for 10 minutes. The end polynucleotides were kinased by mixing 52 μl of the above reaction, 4 μl of a solution containing 10

25   mM ATP and 5 units of T4 polynucleotide kinase. This solution was maintained at 37°C for 30 minutes and then the T4 polynucleotide kinase was inactivated by maintaining the solution at 65°C for 10 minutes.

30   Table 2

       SEQ

       ID NO
       (25)   01)   5' TGAATTCTAAACTAGTCGCCAAGGAGACAGTCAT 3'

       (26)   02)   5' AATGAAATACCTATTGCCTACGGCAGCCGCTGGATT 3'

35   (27)   03)   5' GTTATTACTCGCTGCCCAACCAGCCATGGCC 3'

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(28)   04)   5' GAGCTCGTCAGTTCTAGAGTTAAGCGGCCG 3'
(29)   05)   5' GTATTTCATTATGACTGTCTCCTTGGCGACTAGTTTAGAA-
              TTCAAGCT 3'
(30)   06)   5' CAGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAG 3'
(31)   07)   5' TGACGAGCTCGGCCATGGCTGGTTGGG 3'
(32)   08)   5' TCGACGGCCGCTTAACTCTAGAAC 3'
```

The completed synthetic DNA insert was ligated directly into the Lambda Zap™ II vector described in Example 1a1) that had been previously digested with the restriction enzymes Sac I and Xho I. The ligation mixture was packaged according to the manufacture's instructions using Gigapack II Gold packing extract (Stratagene). The packaged ligation mixture was plated on XL1-Blue cells (Stratagene). Individual lambda plaques were cored and the inserts excised according to the in vivo excision protocol for Lambda Zap™ II provided by the manufacturer (Stratagene). This in vivo excision protocol moved the cloned insert from the Lambda Lc2 vector into a plasmid phagemid vector allow for easy manipulation and sequencing. The accuracy of the above cloning steps was confirmed by sequencing the insert using the manufacture's instructions in the AMV Reverse Transcriptase ^{35}S-dATP sequencing kit (Stratagene). The sequence of the resulting Lc2 expression vector (Lambda Lc2) is shown in Figure 3. Each strand is separately listed in the Sequence Listing as SEQ ID NO 3 and SEQ ID NO 4. The resultant Lc2 vector is schematically diagrammed in Figure 4.

A preferred vector for use in this invention, designated Lambda Lc3, is a derivative of Lambda Lc2 prepared above. Lambda Lc2 contains a Spe I restriction site located 3' to the EcoR I restriction

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site and 5' to the Shine-Dalgarno ribosome binding
site as shown in the sequence in Figure 3 and in SEQ
ID NO 3. A Spe I restriction site is also present in
Lambda Hc2 as shown in Figures 1 and 2 and in SEQ ID
5   NO 1. A combinatorial vector, designated pComb, was
constructed by combining portions of Lambda Hc2 and
Lc2 together as described in Example 1a4) below. The
resultant combinatorial pComb vector contained two Spe
I restriction sites, one provided by Lambda Hc2 and
10   one provided by Lambda Lc2, with an EcoR I site in
between. Despite the presence of two Spe I
restriction sites, DNA homologs having Spe I and EcoR
I cohesive termini were successfully directionally
ligated into a pComb expression vector previously
15   digested with Spe I and EcoR I as described in Example
1b below. The proximity of the EcoR I restriction
site to the 3' Spe I site, provided by the Lc2 vector,
inhibited the complete digestion of the 3' Spe I site.
Thus, digesting pComb with Spe I and EcoR I did not
20   result in removal of the EcoR I site between the two
Spe I sites.

        The presence of a second Spe I restriction site
may be undesirable for ligations into a pComb vector
digested only with Spe I as the region between the two
25   sites would be eliminated. Therefore, a derivative of
Lambda Lc2 lacking the second or 3' Spe I site,
designated Lambda Lc3, was produced by first digesting
Lambda Lc2 with Spe I to form a linearized vector.
The ends were filled in to form blunt ends which are
30   ligated together to result in Lambda Lc3 lacking a Spe
I site. Lambda Lc3 is a preferred vector for use in
constructing a combinatorial vector as described
below.

35   4)   <u>Preparation of pComb</u>

Phagemids were excised from the
expression vectors Lambda Hc2 or Lambda Lc2 using an
in vivo excision protocol described above.   Double
stranded DNA was prepared from the phagemid-containing
cells according to the methods described by Holmes et
al., Anal. Biochem., 114:193 (1981).   The phagemids
resulting from in vivo excision contained the same
nucleotide sequences for antibody fragment cloning and
expression as did the parent vectors, and are
designated phagemid Hc2 and Lc2, corresponding to
Lambda Hc2 and Lc2, respectively.

For the construction of combinatorial phagemid
vector pComb, produced by combining portions of
phagemid Hc2 and phagemid Lc2, phagemid Hc2 was first
digested with Sac I to remove the restriction site
located 5' to the LacZ promoter.   The linearized
phagemid was then blunt ended with T4 polymerase and
ligated to result in a Hc2 phagemid lacking a Sac I
site.   The modified Hc2 phagemid and the Lc2 phagemid
were then separately restriction digested with Sca I
and EcoR I to result in a Hc2 fragment having from 5'
to 3' Sca I, Not I, Xho I, Spe I and EcoR I
restriction sites and a Lc2 fragment having from 5' to
3' EcoR I, Sac I, Xba I and Sac I restriction sites.
The linearized phagemids were then ligated together at
their respective cohesive ends to form pComb, a
circularized phagemid having a linear arrangement of
restriction sites of Not I, Xho I, Spe I, EcoR I, Sac
I, Xba I, Not I, Apa I and Sca I.   The ligated
phagemid vector was then inserted into an appropriate
bacterial host and transformants were selected on the
antibiotic ampicillin.

Selected ampicillin resistant transformants were
screened for the presence of two Not I sites.   The
resulting ampicillin resistant combinatorial phagemid

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vector was designated pComb, the schematic
organization of which is shown in Figure 5. The
resultant combinatorial vector, pComb, consisted of a
DNA molecule having two cassettes to express two

5   fusion proteins and having nucleotide residue
sequences for the following operatively linked
elements listed in a 5' to 3' direction: a first
cassette consisting of an inducible LacZ promoter
upstream from the LacZ gene; a Not I restriction site;

10   a ribosome binding site; a pelB leader; a spacer; a
cloning region bordered by a 5' Xho and 3' Spe I
restriction site; a decapeptide tag followed by
expression control stop sequences; an EcoR I
restriction site located 5' to a second cassette

15   consisting of an expression control ribosome binding
site; a pelB leader; a spacer region; a cloning region
bordered by a 5' Sac I and a 3' Xba I restriction site
followed by expression control stop sequences and a
second Not I restriction site.

20   A preferred combinatorial vector for use in this
invention, designated pComb2, is constructed by
combining portions of phagemid Hc2 and phagemid Lc3 as
described above for preparing pComb. The resultant
combinatorial vector, pComb2, consists of a DNA

25   molecule having two cassettes identical to pComb to
express two fusion proteins identically to pComb
except that a second Spe I restriction site in the
second cassette is eliminated.

30   b.   Construction of the pCombIII Vector for
                  Expressing Fusion Proteins Having a
                  Bacteriophage Coat Protein Membrane Anchor
                  Because of the multiple endonuclease
restriction cloning sites, the pComb phagemid

35   expression vector prepared above is a useful cloning

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vehicle for modification for the preparation of an
expression vector for use in this invention. To that
end, pComb was digested with EcoR I and Spe I followed
by phosphatase treatment to produce linearized pComb.

5

1)   Preparation of pCombIII

A separate phagemid expression vector
was constructed using sequences encoding bacteriophage
cpIII membrane anchor domain. A PCR product defining
10   the DNA sequence encoding the filamentous phage coat
protein,cpIII, membrane anchor containing a LacZ
promotor region sequence 3' to the membrane anchor for
expression of the light chain and Spe I and EcoR I
cohesive termini was prepared from M13mp18, a
15   commercially available bacteriophage vector
(Pharmacia, Piscataway, New Jersey).

To prepare a modified cpIII, replicative form DNA
from M13mp18 was first isolated. Briefly, into 2 ml
of LB (Luria-Bertani medium), 50 µl of a culture of a
20   bacterial strain carrying an F' episome (JM107, JM109
or TG1) was admixed with a one tenth suspension of
bacteriophage particles derived from a single plaque.
The admixture was incubated for 4 to 5 hours at 37°C
with constant agitation. The admixture was then
25   centrifuged at 12,000 x g for 5 minutes to pellet the
infected bacteria. After the supernatant was removed,
the pellet was resuspended by vigorous vortexing in
100 µl of ice-cold solution I. Solution I was
prepared by admixing 50 mM glucose, 10 mM EDTA
30   (disodium ethylenediaminetetraacetic acid) and 25 mM
Tris-HCl at pH 8.0, and autoclaving for 15 minutes.

To the bacterial suspension, 200 µl of freshly
prepared Solution II was admixed and the tube was
rapidly inverted five times. Solution II was prepared
35   by admixing 0.2 N NaOH and 1% SDS. To the bacterial

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suspension, 150 μl of ice-cold Solution III was
admixed and the tube was vortexed gently in an
inverted position for 10 seconds to disperse Solution
III through the viscous bacterial lysate. Solution
5   III was prepared by admixing 60 ml of 5 M potassium
acetate, 11.5 ml of glacial acetic acid and 28.5 ml of
water. The resultant bacterial lysate was then stored
on ice for 5 minutes followed by centrifugation at
12,000 x g for 5 minutes at 4°C in a microfuge. The
10   resultant supernatant was recovered and transferred to
a new tube. To the supernatant was added an equal
volume of phenol/chloroform and the admixture was
vortexed. The admixture was then centrifuged at
12,000 x g for 2 minutes in a microfuge. The
15   resultant supernatant was transferred to a new tube
and the double-stranded bacteriophage DNA was
precipitated with 2 volumes of ethanol at room
temperature. After allowing the admixture to stand at
room temperature for 2 minutes, the admixture was
20   centrifuged to pellet the DNA. The supernatant was
removed and the pelleted replicative form DNA was
resuspended in 25 μl of Tris-HCl at pH 7.6, and 10 mM
EDTA (TE).

       The double-stranded M13mp18 replicative form DNA
25   was then used as a template for isolating the gene
encoding the membrane anchor domain at cpIII, the
sequence of which is listed in the Sequence Listing as
SEQ ID NO 33. The amino acid residue sequence of
membrane anchor domain cpIII is listed in SEQ ID NO
30   34. M13mp18 replicative form DNA was prepared as
described above and used as a template for two PCR
amplifications for construction of a DNA fragment
consisting of the mature gene for cpIII membrane
anchor domain located 5' to a sequence encoding the
35   LacZ promoter, operator and cap-binding site for

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controlling light chain expression. The restriction
sites, Spe I and EcoR I, were created in the
amplification reactions and were located at the 5' and
3' ends of the fragment respectively. The procedure
5   for creating this fragment by combining the products
of two separate PCR amplifications is described below.
        The primer pair, G-3(F) (SEQ ID NO 35) and G-3(B)
(SEQ ID NO 36) listed in Table 3, was used in the
first PCR reaction as performed above to amplify the
10   cpIII membrane anchor gene and incorporate Spe I and
Nhe I restriction sites into the fragment. For the
PCR reaction, 2 µl containing 1 ng of M13mp18
replicative form DNA was admixed with 10 µl of 10X PCR
buffer purchased commercially (Promega Biotech,
15   Madison, Wisconsin) in a 0.5 ml microfuge tube. To
the DNA admixture, 8 µl of a 2.5 mM solution of dNTPs
(dATP, dCTP, dGTP, dTTP) was admixed to result in a
final concentration of 200 micromolar (µM). Three µl
(equivalent to 60 picomoles (pM)) of the G-3(F) primer
20   and 3 µl (60 pM) of the 3' backward G-3(B) primer was
admixed into the DNA solution. To the admixture, 73
µl of sterile water and 1 µl/5 units of polymerase
(Promega Biotech) was added. Two drops of mineral oil
were placed on top of the admixture and 40 rounds of
25   PCR amplification in a thermocycler were performed.
The amplification cycle consisted of 52°C for 2
minutes, 72°C for 1.5 minutes and 91°C for 2 minutes.
The resultant PCR modified cpIII membrane anchor
domain DNA fragment from M13mp18 containing samples
30   were then purified with Gene Clean (BIO101, La Jolla,
California), extracted twice with phenol/chloroform,
once with chloroform followed by ethanol precipitation
and were stored at -70°C in 10 mM Tris-HCl at pH 7.5,
and 1 mM EDTA.
35   The resultant PCR modified cpIII DNA fragment

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having Spe I and Nhe I sites in the 5' and 3' ends, respectively, of the fragment was verified by electrophoresis in a 1% agarose gel. The area in the agarose containing the modified cpIII DNA fragment was

5   isolated from the agarose. The sequence of the PCR modified cpIII membrane anchor domain DNA fragment is listed in the Sequence Listing as SEQ ID NO 40. The resultant amplified PCR fragment also contained nucleotide sequences for encoding a five amino acid

10   tether composed of four glycine residues and one serine juxtaposed between the heavy chain and cpIII encoding domains. Once expressed, the five amino acid residue sequence lacking an orderly secondary structure served to minimize the interaction between

15   the Fab and cpIII domains.

A second PCR amplification using the primer pairs, Lac-F (SEQ ID NO 37) and Lac-B (SEQ ID NO 38) listed in Table 3, was performed on a separate aliquot of M13mp18 replicative form template DNA to amplify

20   the LacZ promoter, operator and Cap-binding site having a 5' Nhe I site and a 3' EcoR I site. The primers used for this amplification were designed to incorporate a Nhe I site on the 5' end of the amplified fragment to overlap with a portion of the 3'

25   end of the cpIII gene fragment and of the Nhe I site 3' to the amplified cpIII fragment. The reaction and purification of the PCR product was performed as described above. The sequence of the resultant PCR modified cpIII DNA fragment having a 5' Nhe I and 3'

30   EcoR I restriction site is listed in the Sequence Listing as SEQ ID NO 41.

An alternative Lac-B primer for use in constructing the cpIII membrane anchor and LacZ promotor region was Lac-B' as shown in Table 3. The

35   amplification reactions were performed as described

above with the exception that in the second PCR
amplification, Lac-B' was used with Lac-F instead of
Lac-B. The product from the amplification reaction is
listed in the sequence listing as SEQ ID NO 41 from
5   nucleotide position 1 to nucleotide position 172. The
use of Lac-B' resulted in a LacZ region lacking 29
nucleotides on the 3' end but was functionally
equivalent to the longer fragment produced with the
Lac-F and Lac-B primers.

10   The products of the first and second PCR
amplifications using the primer pairs G-3(F) and
G-3(B) and Lac-F and Lac-B were then recombined at the
nucleotides corresponding to cpIII membrane anchor
overlap and Nhe I restriction site and subjected to a
15   second round of PCR using the G-3(F) (SEQ ID NO 35)
and Lac-B (SEQ ID NO 38) primer pair to form a
recombined PCR DNA fragment product consisting of the
following: a 5' Spe I restriction site; a cpIII DNA
membrane anchor domain beginning at the nucleotide
20   residue sequence which corresponds to the amino acid
residue 198 of the entire mature cpIII protein; an
endogenous stop site provided by the membrane anchor
at amino acid residue number 112; a Nhe I restriction
site, a LacZ promoter, operator and Cap-binding site
25   sequence; and a 3' EcoR I restriction site.

To construct a phagemid vector for the coordinate
expression of a heavy chain-cpIII fusion protein as
prepared in Example 2 with kappa light chain, the
recombined PCR modified cpIII membrane anchor domain
30   DNA fragment was then restriction digested with Spe I
and EcoR I to produce a DNA fragment for directional
ligation into a similarly digested pComb2 phagemid
expression vector having only one Spe I site prepared
in Example 1a4) to form a pComb2-III (also referred to
35   as pComb2-III) phagemid expression vector. Thus, the

resultant ampicillin resistance conferring pComb2-3
vector, having only one Spe I restriction site,
contained separate LacZ promoter/operator sequences
for directing the separate expression of the heavy
5   chain (Fd)-cpIII fusion product and the light chain
protein. The expressed proteins were directed to the
periplasmic space by pelB leader sequences for
functional assembly on the membrane. Inclusion of the
phage F1 intergenic region in the vector allowed for
10   packaging of single stranded phagemid with the aid of
helper phage. The use of helper phage superinfection
lead to expression of two forms of cpIII. Thus,
normal phage morphogenesis was perturbed by
competition between the Fab-cpIII fusion and the
15   native cpIII of the helper phage for incorporation
into the virion for Fab-cpVIII fusions. In addition,
also contemplated for use in this invention are
vectors conferring chloramphenicol resistance and the
like.
20   A more preferred phagemid expression vector for
use in this invention having additional restriction
enzyme cloning sites, designated pComb-III' or
pComb2-3', was prepared as described above for
pComb2-3 with the addition of a 51 base pair fragment
25   from pBluescript as described by Short et al., Nuc.
Acids Res., 16:7583-7600 (1988) and commercially
available from Stratagene. To prepare pComb2-3',
pComb2-3 was first digested with Xho I and Spe I
restriction enzymes to form a linearized pComb2-3.
30   The vector pBluescript was digested with the same
enzymes releasing a 51 base pair fragment containing
the restriction enzyme sites Sal I, Acc I, Hinc II,
Cla I, Hind III, EcoR V, Pst I, Sma I and BamH I. The
51 base pair fragment was ligated into the linearized
35   pComb2-3 vector via the cohesive Xho I and Spe I

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termini to form pComb2-3'.

Table 3

SEQ
ID NO   Primer

(35)[1]   G-3   (F)   5' GAGACGACTAGTGGTGGCGGTGGCTCTCCATTC
                         GTTTGTGAATATCAA 3'

(36)[2]   G-3   (B)   5' TTACTAGCTAGCATAATAACGGAATACCCAAAA
                         GAACTGG 3'

(37)[3]   LAC-F   5' TATGCTAGCTAGTAACACGACAGGTTTCCCGAC
                         TGG 3'

(38)[4]   LAC-B   5' ACCGAGCTCGAATTCGTAATCATGGTC 3'

(39)5   LAC-B'   5' AGCTGTTGAATTCGTGAAATTGTTATCCGCT 3'

F   Forward Primer

B   Backward Primer

1 From 5' to 3': Spe I restriction site sequence is
single underlined; the overlapping sequence with the
5' end of cpIII is double underlined

2 From 5' to 3': Nhe I restriction site sequence is
single underlined; the overlapping sequence with 3'
end of cpIII is double underlined.

3 From 5' to 3': overlapping sequence with the 3'
end of cpIII is double underlined; Nhe I restriction
sequence begins with the nucleotide residue "G" at
position 4 and extends 5 more residues = GCTAGC.

4 EcoR I restriction site sequence is single
underlined.

5 Alternative backwards primer for amplifying LacZ;
EcoR I restriction site sequence is single underlined.

2.   Isolation of HIV-1-Specific Monoclonal Antibodies
     Produced from the Dicistronic Expression Vector,

<u>pComb2-3</u>

In practicing this invention, the heavy (Fd
consisting of V_H and C_H1) and light (kappa) chains (V_L,
C_L) of antibodies are first targeted to the periplasm
5   of <u>E. coli</u> for the assembly of heterodimeric Fab
molecules. In order to obtain expression of antibody
Fab libraries on a phage surface, the nucleotide
residue sequences encoding either the Fd or light
chains must be operatively linked to the nucleotide
10   residue sequence encoding a filamentous bacteriophage
coat protein membrane anchor. A coat protein for use
in this invention in providing a membrane anchor is
III (cpIII or cp3). In the Examples described
herein, methods for operatively linking a nucleotide
15   residue sequence encoding a Fd chain to a cpIII
membrane anchor in a fusion protein of this invention
are described.

In a phagemid vector, a first and second cistron
consisting of translatable DNA sequences are
20   operatively linked to form a dicistronic DNA molecule.
Each cistron in the dicistronic DNA molecule is linked
to DNA expression control sequences for the coordinate
expression of a fusion protein, Fd-cpIII, and a kappa
light chain.

25   The first cistron encodes a periplasmic secretion
signal (pelB leader) operatively linked to the fusion
protein, Fd-cpIII. The second cistron encodes a
second pelB leader operatively linked to a kappa light
chain. The presence of the pelB leader facilitates
30   the coordinated but separate secretion of both the
fusion protein and light chain from the bacterial
cytoplasm into the periplasmic space.

In this process, the phagemid expression vector
carries an ampicillin selectable resistance marker
35   gene (beta lactamase or bla) in addition to the

Fd-cpIII fusion and the kappa chain. The f1 phage
origin of replication facilitates the generation of
single stranded phagemid. The isopropyl
thiogalactopyranoside (IPTG) induced expression of a

5   dicistronic message encoding the Fd-cpIII fusion (V_H,
C_{H1}, cpIII) and the light chain (V_L, C_L) leads to the
formation of heavy and light chains. Each chain is
delivered to the periplasmic space by the pelB leader
sequence, which is subsequently cleaved. The heavy

10   chain is anchored in the membrane by the cpIII
membrane anchor domain while the light chain is
secreted into the periplasm. The heavy chain in the
presence of light chain assembles to form Fab
molecules. This same result can be achieved if, in

15   the alternative, the light chain is anchored in the
membrane via a light chain fusion protein having a
membrane anchor and heavy chain is secreted via a pelB
leader into the periplasm.

     With subsequent infection of E. coli with a

20   helper phage, as the assembly of the filamentous
bacteriophage progresses, the coat protein III is
incorporated on the tail of the bacteriophage.

     a.   Preparation of Lymphocyte RNA

25   Five milliliters of bone marrow was removed
by aspiration from HIV-1 asymptomatic seropositive
individuals. Total cellular RNA was prepared from the
bone marrow lymphocytes as described above using the
RNA preparation methods described by Chomczynski et

30   al., Anal Biochem., 162:156-159 (1987) and using the
RNA isolation kit (Stratagene) according to the
manufacturer's instructions. Briefly, for immediate
homogenization of the cells in the isolated bone
marrow, 10 ml of a denaturing solution containing 3.0

35   M guanidinium isothiocyanate containing 71 μl of

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beta-mercaptoethanol was admixed to the isolated bone
marrow. One ml of sodium acetate at a concentration
of 2 M at pH 4.0 was then admixed with the homogenized
cells. One ml of phenol that had been previously

5   saturated with H_2O was also admixed to the denaturing
solution containing the homogenized spleen. Two ml of
a chloroform:isoamyl alcohol (24:1 v/v) mixture was
added to this homogenate. The homogenate was mixed
vigorously for ten seconds and maintained on ice for

10   15 minutes. The homogenate was then transferred to a
thick-walled 50 ml polypropylene centrifuged tube
(Fisher Scientific Company, Pittsburgh, PA). The
solution was centrifuged at 10,000 x g for 20 minutes
at 4°C. The upper RNA-containing aqueous layer was

15   transferred to a fresh 50 ml polypropylene centrifuge
tube and mixed with an equal volume of isopropyl
alcohol. This solution was maintained at -20°C for at
least one hour to precipitate the RNA. The solution
containing the precipitated RNA was centrifuged at

20   10,000 x g for twenty minutes at 4°C. The pelleted
total cellular RNA was collected and dissolved in 3 ml
of the denaturing solution described above. Three ml
of isopropyl alcohol was added to the re-suspended
total cellular RNA and vigorously mixed. This

25   solution was maintained at -20°C for at least 1 hour
to precipitate the RNA. The solution containing the
precipitated RNA was centrifuged at 10,000 x g for ten
minutes at 4°C. The pelleted RNA was washed once with
a solution containing 75% ethanol. The pelleted RNA

30   was dried under vacuum for 15 minutes and then
re-suspended in dimethyl pyrocarbonate-treated
(DEPC-H_2O) H_2O.

Messenger RNA (mRNA) enriched for sequences
containing long poly A tracts was prepared from the

35   total cellular RNA using methods described in

Molecular Cloning: A Laboratory Manual, Maniatis et
al., eds., Cold Spring Harbor, NY, (1982). Briefly,
one half of the total RNA isolated from a single donor
prepared as described above was resuspended in one ml
of DEPC-H$_2$O and maintained at 65°C for five minutes.
One ml of 2X high salt loading buffer consisting of
100 mM Tris-HCl, 1 M NaCl, 2.0 mM EDTA at pH 7.5, and
0.2% SDS was admixed to the resuspended RNA and the
mixture allowed to cool to room temperature.

The total purified mRNA was then used in PCR
amplification reactions as described in Example 2c.
Alternatively, the mRNA was further purified to poly
A+ RNA by the following procedure. The total MRNA was
applied to an oligo-dT (Collaborative Research Type 2
or Type 3) column that was previously prepared by
washing the oligo-dT with a solution containing 0.1 M
sodium hydroxide and 5 mM EDTA and then equilibrating
the column with DEPC-H$_2$O. The eluate was collected in
a sterile polypropylene tube and reapplied to the same
column after heating the eluate for 5 minutes at 65°C.
The oligo-dT column was then washed with 2 ml of high
salt loading buffer consisting of 50 mM Tris-HCl at pH
7.5, 500 mM sodium chloride, 1 mM EDTA at pH 7.5 and
0.1% SDS. The oligo dT column was then washed with 2
ml of 1X medium salt buffer consisting of 50 mM
Tris-HCl, pH 7.5, 100 mM, 1 mM EDTA and 0.1% SDS. The
messenger RNA was eluted from the oligo-dT column with
1 ml of buffer consisting of 10 mM Tris-HCl at pH 7.5,
1 mM EDTA at pH 7.5, and 0.05% SDS. The messenger RNA
was purified by extracting this solution with
phenol/chloroform followed by a single extraction with
100% chloroform. The messenger RNA was concentrated
by ethanol precipitation and resuspended in DEPC H$_2$O.

The resultant purified mRNA contained a plurality
of anti-HIV encoding V$_H$ and V$_L$ sequences for

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preparation of an anti-HIV-1 Fab DNA library.

    b.   <u>Construction of a Combinatorial HIV-1
Antibody Library</u>

5   1)   <u>Selection of Oligonucleotide Primers</u>

The nucleotide sequences encoding the
immunoglobulin protein CDR's are highly variable.
However, there are several regions of conserved
sequences that flank the V region domains of either
10 the light or heavy chain, for instance, and that
contain substantially conserved nucleotide sequences,
i.e., sequences that will hybridize to the same primer
sequence. Therefore, polynucleotide synthesis
(amplification) primers that hybridize to the
15 conserved sequences and incorporate restriction sites
into the DNA homolog produced that are suitable for
operatively linking the synthesized DNA fragments to a
vector were constructed. More specifically, the
primers were designed so that the resulting DNA
20 homologs produced can be inserted into an expression
vector of this invention in reading frame with the
upstream translatable DNA sequence at the region of
the vector containing the directional ligation means.

For amplification of the V_H domains, primers were
25 designed to introduce cohesive termini compatible with
directional ligation into the unique Xho I and Spe I
sites of the pComb2-3 expression vector. In all
cases, the 5' primers VH1a (5'
CAGGTGCAG<u>CTCGAG</u>CAGTCTGGG 3' SEQ ID NO 42) and VH3a (5'
30 GAGGTGCAG<u>CTCGAG</u>GAGTCTGGG 3' SEQ ID NO 43) were
designed to maximize homology with the V_H1 and V_H3
subgroup families, respectively, although considerable
cross-priming of other subgroups was expected. The
Xho I restriction site for cloning into the pComb2-3
35 vector is underlined. The 3' primer CG1z having the

nucleotide sequence 5' GCATGT<u>ACTAGT</u>TTTGTCACAAGATTTGGG
3' (SEQ ID NO 44) used in conjunction with the 5'
primers is the primer for the heavy chain
corresponding to part of the hinge region. The Spe I
5   site for cloning into the pComb2-3 vector is
underlined.

    The nucleotide sequences encoding the V_L domain
are highly variable. However, there are several
regions of conserved sequences that flank the V_L
10   domains including the J_L, V_L framework regions and V_L
leader/promotor. Therefore, amplification primers
were constructed that hybridized to the conserved
sequences and incorporate restriction sites that allow
cloning the amplified fragments into the pComb2-3
15   expression vector cut with Sac I and Xba I.

    For amplification of the kappa V_L domains
analogous to the heavy chain primers listed above, the
5' primers, VK1a (5' GACATC<u>GAGCTC</u>ACCCAGTCTCCA 3' SEQ
ID NO 45) and VK3a (5' GAAATT<u>GAGCTC</u>ACGCAGTCTCCA 3' SEQ
20   ID NO 46), were used. These primers also introduced a
Sac I restriction endonuclease site indicated by the
underlined nucleotides to allow the V_L DNA homolog to
be cloned into the pComb2-3 expression vector. The 3'
V_L amplification primer, CK1a having a nucleotide
25   sequence 5'
GCGCCG<u>TCTAGA</u>ACTAACACTCTCCCCTGTTGAAGCTCTTTGTGACGGGCAAG
3' (SEQ ID NO 47) corresponding to the 3' end of the
light chain was used to amplify the light chain while
incorporating the underlined Xba I restriction
30   endonuclease site required to insert the V_L DNA
homolog into the pComb2-3 expression vector.

    All primers and synthetic polynucleotides
described herein, were either purchased from Research
Genetics in Huntsville, Alabama or synthesized on an
35   Applied Biosystems DNA synthesizer, model 381A, using

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the manufacturer's instruction.

    2)   PCR Amplification of V$_H$ and V$_L$ DNA
          Homologs

          In preparation for PCR amplification,
mRNA prepared above was used as a template for cDNA
synthesis by a primer extension reaction. First,
20-50 μg of total mRNA in water was first hybridized
(annealed) at 70°C for 10 minutes with 600 ng (60.0
pmol) of either the heavy or light chain 3' primers
listed above. Subsequently, the hybridized admixture
was used in a typical 50 μl reverse transcription
reaction containing 200 μM each of dATP, dCTP, dGTP
and dTTP, 40 mM Tris-HCl at pH 8.0, 8 mM MgCl$_2$, 50 mM
NaCl, 2 mM spermidine and 600 units of reverse
transcriptase (SuperScript, BRL). The reaction
admixture was then maintained for one hour at 37°C to
form an RNA-cDNA admixture.

          Three μl of the resultant RNA-cDNA admixture was
then used in PCR amplification in a reaction volume of
100 μl containing a mixture of all four dNTPs at a
concentration of 60 μM, 50 mM KCl, 10 mM Tris-HCl at
pH 8.3, 15 mM MgCl$_2$, 0.1% gelatin and 5 units of
Thermus aquaticus (Taq) DNA polymerase (Perkin-Elmer-
Cetus, Emeryville, California), and 60 pmol of the
appropriate 5' and 3' primers listed above. The
separate reaction admixtures were overlaid with
mineral oil and subjected to 35 cycles of
amplification. Each amplification cycle included
denaturation at 91°C for 1 minute, annealing at 52°C
for 2 minutes and polynucleotide synthesis by primer
extension (elongation) at 72°C for 1.5 minutes,
followed by a final maintenance period of 10 minutes
at 72°C. An aliquot of the reaction admixtures were
then separately electrophoresed on a 2% agarose gel.

After successful amplification as determined by gel
electrophoretic migration, the remainder of the
RNA-cDNA was amplified after which the PCR products of
a common 3' primer were pooled into separate V_H-and

5   V_L-coding DNA homolog-containing samples and were then
extracted twice with phenol/chloroform, once with
chloroform, ethanol precipitated and were stored at
-70°C in 10 mM Tris-HCl at pH 7.5, and 1 mM EDTA.

10   3)   <u>Insertion of V_H and V_L-Coding DNA</u>
                          <u>Homologs into pComb2-3 Expression</u>
                          <u>Vector</u>
                          The V_H-coding DNA homologs (heavy
chain) prepared above were then digested with an

15   excess of Xho I and Spe I for subsequent ligation into
a similarly digested and linearized pComb2-3 in a
total volume of 150 μl with 10 units of ligase at 16°C
overnight. The construction of the library was
performed as described by Burton et al., <u>Proc. Natl.</u>

20   <u>Acad. Sci., USA</u>, 88:10134-10137 (1991). Briefly,
following ligation, the pComb2-3 vector containing
heavy chain DNA was then transformed by
electroporation into 300 μl of XL1-Blue cells. After
transformation and culturing, library size was

25   determined by plating aliquots of the culture.
Typically the library had about 10^7 members. An
overnight culture was then prepared from which
phagemid DNA containing the heavy chain library was
prepared.

30   For the cloning of the V_L-coding DNA homologs
(light chain), 10 μg of phagemid DNA containing the
heavy chain library was then digested with Sac I and
SbaI. The resulting linearized vector was treated
with phosphatase and purified by agarose gel

35   electrophoresis. The desired fragment, 4.7 kb in

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length, was excised from the gel. Ligation of this
vector with prepared light chain PCR DNA proceeded as
described above for heavy chain. A library of
approximately 10^7 members having heavy chain fragments
5   operatively linked to the cpIII anchor sequence
(Fd-cpIII) and light chain fragments was thus
produced.

                    4)   Preparation of Phage Expressing Fab
10   Heterodimers
                          Following transformation of the
resultant library produced above into XL1-Blue cells,
phage were prepared to allow for isolation of HIV-1
specific Fabs by panning on target antigens. To
15   isolate phage on which heterodimer expression has been
induced, 3 ml of SOC medium (SOC was prepared by
admixture of 20 g bacto-tryptone, 5 g yeast extract
and 0.5 g NaCl in one liter of water, adjusting the pH
to 7.5 and admixing 20 ml of glucose just before use
20   to induce the expression of the Fd-cpIII and light
chain heterodimer) was admixed and the culture was
shaken at 220 rpm for one hour at 37°C, after which
time 10 ml of SB (SB was prepared by admixing 30 g
tryptone, 20 g yeast extract, and 10 g Mops buffer per
25   liter with pH adjusted to 7) containing 20 μg/ml
carbenicillin and 10 μg/ml tetracycline and the
admixture was shaken at 300 rpm for an additional
hour. This resultant admixture was admixed to 100 ml
SB containing 50 μg/ml carbenicillin and 10 μg/ml
30   tetracycline and shaken for one hour, after which time
helper phage VCSM13 (10^{12} pfu) were admixed and the
admixture was shaken for an additional two hours.
After this time, 70 μg/ml kanamycin was admixed and
maintained at 30°C overnight. The lower temperature
35   resulted in better heterodimer incorporation on the

surface of the phage. The supernatant was cleared by
centrifugation (4000 rpm for 15 minutes in a JA10
rotor at 4°C). Phage were precipitated by admixture
of 4% (w/v) polyethylene glycol 8000 and 3% (w/v) NaCl

5   and maintained on ice for 30 minutes, followed by
centrifugation (9000 rpm for 20 minutes in a JA10
rotor at 4°C). Phage pellets were resuspended in 2 ml
of PBS and microcentrifuged for three minutes to
pellet debris, transferred to fresh tubes and stored

10   at -20°C for subsequent screening as described below.

For determining the titering colony forming units
(cfu), phage (packaged phagemid) were diluted in SB
and 1 μl was used to infect 50 μl of fresh (OD600 = 1)
XL1-Blue cells grown in SB containing 10 μg/ml

15   tetracycline. Phage and cells were maintained at room
temperature for 15 minutes and then directly plated on
LB/carbenicillin plates.

5)   Selection of Anti-HIV-1 Heterodimers on
20   Phage Surfaces
(a)   Multiple Pannings of the Phage
Library
The phage library produced in
Example 2b4) was panned against recombinant gp120 of

25   HIV-1 strain IIIb as described herein on coated
microtiter plate to select for anti-gp120
heterodimers. A second phage library was panned
against recombinant gp41 (American Biotechnologies,
Boston, MA) as described below to select for anti-gp41

30   heterodimers.
The panning procedure used was a modification of
that originally described by Parmley and Smith
(Parmley et al., Gene, 73:305-318 (1988). Four rounds
of panning were performed to enrich for specific

35   antigen-binding clones. For this procedure, four

wells of a microtiter plate (Costar 3690) were coated
overnight at 4°C with 25 μl of 40 μg/ml gp120 or gp41
(American Biotechnologies) prepared above in 0.1 M
bicarbonate, pH 8.6. The wells were washed twice with

5   water and blocked by completely filling the well with
3% (w/v) BSA in PBS and maintaining the plate at 37°C
for one hour. After the blocking solution was shaken
out, 50 μl of the phage library prepared above
(typically 10^{11} cfu) were admixed to each well, and

10   the plate was maintained for two hours at 37°C.

       Phage were removed and the plate was washed once
with water. Each well was then washed ten times with
TBS/Tween (50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 0.5%
Tween 20) over a period of one hour at room

15   temperature where the washing consisted of pipetting
up and down to wash the well, each time allowing the
well to remain completely filled with TBS/Tween
between washings. The plate was washed once more with
distilled water and adherent phage were eluted by the

20   addition of 50 μl of elution buffer (0.1 M HCl,
adjusted to pH 2.2 with solid glycine, containing 1
mg/ml BSA) to each well followed by maintenance at
room temperature for 10 minutes. The elution buffer
was pipetted up and down several times, removed, and

25   neutralized with 3 μl of 2 M Tris base per 50 μl of
elution buffer used.

       Eluted phage were used to infect 2 ml of fresh
(OD_{600} = 1) E. coli XL1-Blue cells for 15 minutes at
room temperature, after which time 10 ml of SB

30   containing 20 μg/ml carbenicillin and 10 μg/ml
tetracycline was admixed. Aliquots of 20, 10, and
1/10 μl were removed from the culture for plating to
determine the number of phage (packaged phagemids)
that were eluted from the plate. The culture was

35   shaken for one hour at 37°C, after which it was added

to 100 ml of SB containing 50 μg/ml carbenicillin and 10 μg/ml tetracycline and shaken for one hour. Helper phage VCSM13 (10^{12} pfu) were then added and the culture was shaken for an additional two hours. After this time, 70 μg/ml kanamycin was added and the culture was incubated at 37°C overnight. Phage preparation and further panning were repeated as described above.

Following each round of panning, the percentage yield of phage were determined, where % yield - (number of phage eluted/number of phage applied) X 100. The initial phage input ratio was determined by titering on selective plates to be approximately 10^{11} cfu for each round of panning. The final phage output ratio was determined by infecting two ml of logarithmic phase XL1-Blue cells as described above and plating aliquots on selective plates. In the first panning for gp120-reactive phage, 4.6 X 10^{11} phage were applied to four wells and 7.7 X 10^{5} phage were eluted. After the fourth panning 1.0 X 10^{8} phage were eluted. From this procedure, 20 clones were selected from the Fab library for their ability to bind to glycosylated recombinant gp120 from the IIIB strain of HIV-1. Five clones were selected from the Fab library specific for binding to gp41. The panned phage surface libraries were then converted into ones expressing soluble Fab fragments for further screening by ELISA as described below.

In addition to panning on gp120 of strain IIIB and gp41, also contemplated as antigens for panning of combinatorial libraries is recombinant gp120 (IIIB strain) produced in baculovirus and recombinant gp120 (SF2 strain) produced in Chinese Hamster Ovary cells obtained as described by Steimer et al., Science, 254:105-108 (1991). Another antigen, a synthetic

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cyclic peptide, N=CH-(CH$_2$)$_3$CO[SISGPGRAFYTG]NCH$_2$CO-Cys-
NH$_2$ (SEQ ID NO 48) prepared as described by
Satterthwait et al., <u>Bulletin of the World Health
Organization</u>, 68: Suppl., 17-25 (1990) corresponding

5   to the central most conserved part of the V3 loop of
gp120 was coupled to maleimide-activated BSA. The
library was panned using 1, 2 or 4 ELISA wells coated
with 1 μg of protein antigen or 10 μg BSA-peptide per
well. Four rounds of panning were carried out for

10   each antigen as described above. Eluted phage from
the final round were used to infect XL1-Blue cells.
Four rounds of panning against the four antigens
produced an amplification in eluted phage of between
100 and 1000 fold. The panned phage surface libraries

15   were then converted into ones expressing soluble Fab
fragments for further screening by ELISA as described
below.

        6)   <u>Preparation of Soluble Heterodimers and</u>
20   <u>Characterization of Binding Specificity</u>
               <u>to HIV-1 Antigens</u>
               In order to further characterize the
specificity of the mutagenized heterodimers expressed
on the surface of phage as described above, soluble

25   Fab heterodimers from acid eluted phage were prepared
and analyzed in ELISA assays on HIV-1 derived
antigen-coated plates and by competitive ELISA.
        To prepare soluble heterodimers, phagemid DNA
from the 20 gp120 positive clones and the 5 gp41

30   positive clones prepared above was isolated and
digested with Spe I and Nhe I. Digestion with these
enzymes produced compatible cohesive ends. The 4.7 kb
DNA fragment lacking the gene III portion was
gel-purified (0.6% agarose) and self-ligated.

35   Transformation of <u>E. coli</u> XL1-Blue afforded the

isolation of recombinants lacking the cpIII fragment. Clones were examined for removal of the cpIII fragment by Xho I - Xba I digestion, which should yield an 1.6-kb fragment. Clones were grown in 100 ml SB

5   containing 50 μg/ml carbenicillin and 20 mM MgCl$_2$ at 37°C until an OD$_{600}$ of 0.2 was achieved. IPTG (1 mM) was added and the culture grown overnight at 30°C (growth at 37°C provides only a light reduction in heterodimer yield). Cells were pelleted by

10   centrifugation at 4000 rpm for 15 minutes in a JA10 rotor at 4°C. Cells were resuspended in 4 ml PBS containing 34 μg/ml phenylmethylsulfonyl fluoride (PMSF) and lysed by sonication on ice (2-4 minutes at 50% duty). Debris was pelleted by centrifugation at

15   14,000 rpm in a JA20 rotor at 4°C for 15 minutes. The supernatant was used directly for ELISA analysis as described below and was stored at -20°C. For the study of a large number of clones, 10 ml cultures provided sufficient heterodimer for analysis. In this

20   case, sonications were performed in 2 ml of buffer.
        Assays as described above were also performed for the gp41-specific clones.

                    a)   Screening by ELISA

25   The soluble heterodimers prepared above were assayed by ELISA. For this assay, gp120 and gp41 were separately admixed to individual wells of a microtiter plate as described above for the panning procedure and maintained at 4°C overnight to

30   allow the protein solution to adhere to the walls of the well. After the maintenance period, the wells were washed five times with water and thereafter maintained for one hour at 37°C with 100 μl solution of 1% BSA diluted in PBS to block nonspecific sites on

35   the wells. Afterwards, the plates were inverted and

shaken to remove the BSA solution. Twenty-five μl of
soluble heterodimers prepared above reactive with the
specific glycoprotein substrate were then admixed to
each well and maintained at 37°C for one hour to form

5   immunoreaction products. Following the maintenance
period, the wells were washed ten times with water to
remove unbound soluble antibody and then maintained
with a 25 μl of a 1:1000 dilution of secondary goat
anti-human IgG F(ab')$_2$ conjugated to alkaline

10   phosphatase diluted in PBS containing 1% BSA. The
wells were maintained at 37°C for one hour after which
the wells were washed ten times with water followed by
development with 50 μl of p-nitrophenyl phosphate
(PNPP). Color development was monitored at 405 nm.

15   Positive clones gave A405 values of >1 (mostly >1.5)
after 10 minutes, whereas negative clones gave values
of 0.1 to 0.2.

       Approximate concentrations of gp120-reactive Fab
were determined by ELISA using a sandwich ELISA as

20   described by Zebedee et al., Proc. Natl. Acad. Sci.,
USA, 89:3175-3179 (1992) and are presented in the
first column of Figure 6. In addition, since Fabs are
expressed in E. coli and the fraction of correctly
assemble protein can vary, the amount of Fab reacting

25   with gp120 was also assessed by ELISA titration. That
data is also presented in Figure 6 in the second
column.

       For the clones panned against the HIV-1 derived
antigens, after conversion of the panned phage surface

30   libraries to ones expressing soluble Fab fragments,
30-40 colonies were used to transform XL1-Blue cells
and the supernates screened in ELISA assays against
the antigen used in panning. Generally greater than
80% of the supernates tested positive. A

35   representative number of positives were then selected

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from each antigen panning for further analysis.

        (b)   <u>Competitive ELISA with Soluble</u>
               <u>gp120 and CD4</u>

5   Immunoreactive heterodimers as
determined in the above ELISA were then analyzed by
competition ELISA to determine the affinity of the
selected heterodimers. The ELISA was performed as
described above on microtiter wells separately coated
10   with 5 μg/ml of gp120 or soluble CD4 (American
Biotechnologies) in 0.1 M bicarbonate buffer at pH
8.6. Increasing concentrations of soluble or free
gp120 ranging in concentration from 10^{-11} M up to 10^{-7} M
diluted in 0.5% BSA/0.025% Tween 20/PBS were admixed
15   with soluble heterodimers, the dilutions of which were
determined in titration experiments that resulted in
substantial reduction of OD values after a 2-fold
dilution. For the CD4 competition assays, increasing
concentrations of soluble or free CD4 ranging in
20   concentration from 10^{-11} M up to 10^{-6} M diluted in 0.5%
BSA/0.025% Tween 20/PBS were admixed with soluble
heterodimers. The plates were maintained for 90-120
minutes at 37°C and carefully washed ten times with
0.05% Tween 20/PBS before admixture of alkaline
25   phosphatase-labelled goat anti-human IgG F(ab')2 at a
dilution of 1:500 followed by maintenance for 1 hour
at 37°C. Development was performed as described for
ELISA.

           To establish the relationship between
30   neutralizing ability as described in Example 3 below
could be related to antigen binding affinity of
HIV-1-specific Fabs, competition ELISAs were carried
out where soluble gp120 was competed with gp120 coated
on ELISA plates for Fab binding. Figure 7 shows that
35   all Fabs were competed from binding to gp120 with a

IC_{50} of approximately 10^{-9} M free gp120. In addition
as shown in Example 3, there is no correlation between
antigen affinity and neutralization. The Fabs tested
included Fabs 4, 12, 21 and 7 that are members of the
5   same groups as determined by sequence analysis and
comparison as described in Example 4. Fabs 13, 27, 6,
29, 2 and 3 are all members of the different groups as
determined by sequence analysis and comparison as
described in Example 4. Loop 2 is an Fab fragment
10   selected from the same library as the other Fabs but
which recognizes the V3 loop. Only with the V3 loop
peptide was competition carried out with gp120 from
the SF2 strain.

        To investigate whether neutralization could be
15   associated with blocking of the gp120-CD4 interaction,
competition ELISAs were carried out with soluble CD4
competing with Fabs for binding to gp120-coated ELISA
wells. The results are shown in Figure 8. P4D10 and
loop 2 are controls not expected to be competed by
20   CD4. P4D10 is a mouse monoclonal antibody reacting
with the V3 loop of gp120 (IIIB). Loop 2 Fab
competition was carried out using gp120 (SF2). As
shown in Figure 8 the binding of all Fabs with the
exception of the controls was inhibited with an IC_{50}
25   of approximately 10^{-8} M of soluble CD4. In addition,
no difference was detected between the neutralizing
and non-neutralizing Fabs to gp120 inhibited by CD4.
This implies that blocking of the CD4-gp120
interaction is unlikely to be an important factor in
30   Fab neutralization of the HIV-1 virus.

        Similar competition assays were performed with
the Fabs panned against the four HIV-1 derived
antigens. The 19 Fabs derived from panning against
gp120 (IIIB) showed apparent affinities
35   (1/concentration at 50% inhibition) for gp120 (IIIB)

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in the range 10^7 - 10^{-9} M with most being 1-3 X 10^{-8} M.
The panning procedure tends to select strongly for
tight binders so a grouping into a relatively narrow
band of affinities was expected. Of 16 Fabs derived

5   from panning against gp160 (IIIB), 6 were also
reactive with gp120 (IIIB) and competition ELISAs
showed they had similar apparent affinities as the
gp120-panned Fabs. The non-gp120 reactive clones from
the gp160 panning showed a lower ELISA reactivity with

10   gp160 and could not be satisfactorily competed with
gp160. They may be directed against gp41 but were not
pursued here. Eight Fabs derived from panning against
gp120 (SF2) also showed strong ELISA reactivity with
gp120 (IIIB) and gave similar apparent binding

15   affinities. Four Fabs were derived from panning
against the V3 loop peptide. Of these Fabs, 2 reacted
in ELISA with gp120 (SF2) but none with gp120 (IIIB).
The apparent binding affinity of these loop binders to
gp120 (SF2) was 10^{-8} M.

20   To complete the survey in terms of strain
cross-reactivity of Fabs, those derived from the gp120
and gp160 (IIIB) pannings were examined for ELISA
reactivity with gp120 (SF2). All were reactive.
Therefore, all the Fabs examined, with the exception

25   of those selected by panning against the V3 loop
peptide, bound to gp120 from IIIB and SF2 strains.
The Fabs were screened for CD4 inhibition of
their binding to gp120 (IIIB) immobilized on ELISA
wells. All, again with the exception of the V3 loop

30   binders, showed sensitivity to CD4 inhibition. The
inhibition constants were in the range 10^{-7} to 10^{-9} M.
Also contemplated is a competition ELISA assay
where the binding of HIV-1 recombinant Fabs of this
invention is performed in the presence of excess Fabs

35   of this invention as well as those HIV-1 antibodies,

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polyclonal or monoclonal, present in patient sera,
either asymptomatic or symptomatic, or obtained by
other means such as EBV transformation and the like.
The ability of an exogenously admixed antibody to
5   compete for the binding of a characterized Fab of this
invention will allow for the determination of
equivalent antibodies in addition to unique epitopes
and binding specificities.

10   3.   Neutralizing Activity of Recombinant Human Fab
              Fragments Against HIV-1 In Vitro
              Binding of antibodies to viruses can result in
loss of infectivity or neutralization and, although
not the only defense mechanism against viruses, it is
15   widely accepted that antibodies have an important role
to play. However, understanding of the molecular
principles underlying antibody neutralization is
limited and lags behind that of the other effector
functions of antibody. Such understanding is required
20   for the rational design of vaccines and for the most
effective use of passive antibody for prophylaxis or
therapy. This is particularly urgent for the human
immunodeficiency viruses.
              A number of studies have led to the general
25   conclusion that viruses are neutralized by more than
one mechanism and the one employed will depend on
factors such as the nature of the virus, the epitope
recognized, the isotype of the antibody, the cell
receptor used for viral entry and the virus:antibody
30   ratio. The principle mechanisms of neutralization can
be considered as aggregation of virions, inhibition of
attachment of virus to cell receptor and inhibition of
events following attachment such as fusion of viral
and cellular membranes and secondary uncoating of the
35   virion. One of the important features of the third

mechanism is that it may require far less than the
approximately stoichiometric amounts of antibody
expected for the first two mechanisms since occupation
of a small number of critical sites on the virion may
be sufficient for neutralization. For instance it has
been shown that neutralization of the influenza A
virion obeys single hit kinetics as described by
Outlaw et al., _Epidemiol. Infect._, 106:205-220 (1992).

Intensive studies have been carried out on
antibody neutralization of HIV-1. For review, see
Nara et al., _FASEB J._, 5:2437-2455 (1991). Most have
focussed on a single linear epitope in the third
hypervariable domain of the viral envelope
glycoprotein gp120 known as the V3 loop. Antibodies
to this loop are suggested to neutralize by inhibiting
fusion of viral and cell membranes. Binding to the
loop resulting in neutralization can occur prior to
virus-cell interaction or following gp120 binding to
CD4. See, Nara, In Retroviruses of Human Aids and
Related Animal Diseases, eds. Girard et al., pp.
138-150 (1988); Linsely et al., _J. Virol._,
62:3695-3702 (1988); and Skinner et al., _J. Virol._,
67:4195-4200 (1988). Features of the V3 loop are
sequence variability within the loop [Goudsmit et al.,
FASEB J., 5:2427-2436 (1991) and Albert et al., _AIDS_,
4:107-112 (1990)] and sensitivity of neutralizing
antibodies against the loop to sequence variations
outside the loop [Nara et al., _FASEB J._, 5:2437-2455
(1991); Albert et al., _supra_; McKeating et al., _AIDS_,
3:777-784 (1989); and Wahlberg et al., _AIDS Res. Hum._
Retroviruses, 7:983-990 (1991). Hence anti-V3 loop
antibodies are often strain specific and mutations in
the loop _in vivo_ may provide a mechanism for viral
escape from antibody neutralization.

Recently considerable interest has focused on

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antibodies capable of blocking CD4 binding to gp120.
A number of groups have described the features of
these antibodies as (a) reacting with conformational
i.e., non-linear epitopes, (b) reacting with a wide
5   range of virus isolates and (c) being the predominant
neutralizing antibodies in humans after longer periods
of infection. See, Berkower,et al., J. Virol.,
65:5983-5990 (1991); Steimer et al., Science,
254:105-108 (1991); Ho et al., J. Virol., 65:489-493
10   (1991); Kang et al., Proc. Natl. Acad. Sci., USA,
88:6171-6175 (1991); Posner et al., J. Immunol.,
146:4325-4332 (1991); and Tilley et al., Res. Virol.,
142:247-259 (1991). Neutralizing antibodies of this
type would appear to present a promising target for
15   potential therapeutics. The mechanism(s) of
neutralization of these antibodies is unknown although
there is some indication that this may not be blocking
of virus attachment since a number of mouse monoclonal
antibodies inhibiting CD4 binding to gp120 are either
20   non-neutralizing or only weakly neutralizing.

The generation of human monoclonal antibodies
against the envelope of HIV-1 as described by Burton
et al., Proc. Natl. Acad. Sci., USA, 88:10134-10137
(1991) using combinatorial libraries allows a novel
25   approach to the problem of neutralization. Given the
lack of a three-dimensional structure for gp120 and
the complexity of the virus, the approach seeks to
explore neutralization at the molecular level through
the behavior of related antibodies. This is possible
30   for the following reasons: (1) the combinatorial
approach allows the rapid generation of large numbers
of human antibodies; (2) the antibodies (Fab
fragments) are expressed in E.coli and can readily be
sequenced; and (3) antibodies have similar sequences
35   and common structural motifs allowing functional

differences to be meaningfully correlated with primary
structure.

Neutralization studies were performed as
described herein on the human recombinant Fab
fragments from 20 clones against gp120 prepared as
described in Examples 1 and 2, all of which are strain
cross-reactive and inhibited by CD4 from binding to
gp120. The results presented herein show that
neutralization was not effected by virus aggregation
or cross-linking of gp120 molecules on the virion
surface and was not correlated with blocking of the
interaction between soluble CD4 and recombinant gp120.

Neutralization studies were also performed as
described herein on the human recombinant Fab
fragments from the gp41-reactive clones prepared as
described in Examples 1 and 2. The results are
presented below.

Two different assays, a p24 ELISA assay and a
syncytium assay, were performed to measure
neutralization ability of the recombinant human HIV-1
immunoreactive Fabs. An additional assay, a plaque
assay, was performed for determining the
neutralization effectiveness of the gp41-reactive
Fabs. In plaque assays, CD4+ cells were cultured int
he presence or absence of soluble gp41-reactive Fabs
prior to inoculation with virus. Inhibition of
infectivity, also referred to as neutralization, by
antibodies was expressed as the percent of plaque
formation in the cultures compared to cells exposed to
PBS alone.

For some of these assays, the recombinant Fabs
were first purified. One liter cultures of SB
containing 50 μg/ml carbenicillin and 20 mM MgCl$_2$ were
inoculated with appropriate clones and induced 7 hours
later with 2 mM IPTG and grown overnight at 30°C. The

cell pellets were sonicated and the resultant
supernatant were concentrated to a 50 ml volume. The
filtered supernatants were loaded on a 25 ml protein
G-anti-Fab column, washed with 120 ml buffer at a rate

5   of 3 ml/minute and eluted with citric acid at pH 2.3.
The neutralized fractions were then concentrated and
exchanged into 50 mM MES at pH 6.0 and loaded onto a 2
ml Mono-S column at a rate of 1 ml/minute. A gradient
of 0-500 mM NaCl was run at 1 ml/minute with the Fab

10   eluting in the range of 200-250 mM NaCl. After
concentrating, the Fabs were positive when titered on
ELISA against gp120 and gave a single band at 50 kD by
10-15% SDS-PAGE. Concentration was determined by
absorbance measurement at 280nm using an extinction

15   coefficient (1 mg/ml) of 1.4.

        a.   Neutralization as Measured by the p24 ELISA
              Assay

              For this assay, diluted tissue culture

20   supernatants of HIV-1 IIIB or MN-infected peripheral
blood mononuclear cells (PBMC) ($50TCID_{50}$ (50% tissue
culture infectious dose), 100 μl) were maintained for
2 hours at 37°C with serial dilutions (1:2), beginning
at a dilution of 1:20, of recombinant Fab supernates

25   prepared in Example 2b6). Control Fab supernates were
also provided that included human neutralizing sera, a
known human neutralizing monoclonal antibody 2F5 and
the Fab fragment derived from that antibody by papain
digestion, and a known mouse neutralizing monoclonal

30   antibody and its $F(ab')_2$ fragment as described by
Broliden et al., J. Virol., 64:936-940 (1990). PBMC
(1×10^5 cells) were admixed to the virus/antibody
admixture and maintained for 1 hour at 37°C.
Thereafter, the cells were washed and maintained in

35   RPMI 1640 medium (GIBCO) supplemented with 10% fetal

calf serum, 1% glutamine, antibiotics and IL-2. The
culture medium was changed at days 1 and 4. At 7 days
post-infection, supernates were collected and analyzed
by HIV-1 p24 antigen capture ELISA as described by

5   Sundqvist et al., J. Med. Virol., 29:170-175 (1989)
the disclosure of which is hereby incorporated by
reference. Neutralization was defined as positive if
an 80% or greater reduction of optical density at
490nm in the culture supernatant occurred as compared

10   to negative Fab or negative human serum. Tests with
all Fabs, mAbs and sera were repeated on at least two
occasions.

        b.   Quantitative Infectivity Assay Based on
15   Syncytial Formation
              A quantitative neutralization assay with the
MN strain of HIV-1 was performed as described by Nara
et al., AIDS Res. Human Retroviruses, 3:283-302
(1987), the disclosure of which is hereby incorporated

20   by reference. Monolayers of CEM-SS target cells were
cultured with virus, in the presence or absence of
antibody, and the number of syncytia forming units
determined 3-5 days later. An equivalent amount of
virus was used in the assays to allow direct

25   comparison of the various antibody concentrations
tested. The assays were repeatable over a virus-
surviving fraction range of 1 to 0.001 within a 2 to
4-fold difference in the concentration of antibody
(P<0.001).

30

        c.   Results of the Neutralization Assays for
              gp120
              Assays were generally repeated at least
twice with reproducible results. For the data

35   reported in Figure 6, the gp120-specific Fab

supernates were divided into two parts, one being used in the p24 assay and the other in the syncytia assay. A dash (-) indicates that there was no neutralization at 1:20 dilution in the p24 assay and 1:16 in the

5   syncytial assay (with most clones showing no detectable neutralization at a 1:4 dilution). Neutralization titers are indicated in the figure. For the p24 assay, the titer corresponds to the greatest dilution producing >80% reduction in

10   absorbance in ELISA. For the syncytia assay, Fabs 4 and 12 produced >95% neutralization at a 1:4 dilution of supernate and 80 and 70% reduction at 1:128 dilution respectively. These Fabs were effective neutralizers in both types of assays. They have also

15   been shown to neutralize infection by IIIB and RF strains using a PCR-based assay of proviral integration. Fabs 6 and 7 showed no neutralization in the syncytia assay but other supernate preparations showed activity. Fab 13 was consistently effective in

20   the p24 assay but not in the syncytia assay. A number of other clones show lower levels of neutralizing ability.

       Fabs were purified from a selection of some of the clones as described above and used in both

25   neutralization assays. As shown in Figure 9, Fabs 4 and 12 were again effective in both assays at similar levels with for example 50% inhibition of syncytial formation at an Fab concentration of approximately 20 nM (1 μg/ml). The results shown are derived from the

30   syncytia assay using the MN strain. Fabs 7 and 21 were equally effective in the syncytial assay but somewhat less so in the p24 assay. The p24 assay indicated greater than 80% neutralization of HIV-1 MN strain for Fab 4 at 3, Fab 7 at 15, Fab 12 at 3, Fab

35   13 at 4 and Fab 21 at 7 μg/ml, respectively. Fab 13

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however was ineffective in the syncytial assay at 25 μg/ml. For the IIIB strain, greater than 80% neutralization was observed for Fab 4 at 13, Fab 7 at 15, Fab 12 at 7 and Fab 21 at 14 μg/ml, respectively.

5   Although Fab 11 was not effective in neutralization assays when unpurified as shown in Figure 6, following purification, Fab 11 was equally effective as Fab 12 in neutralizing HIV-1. For this reason, the Fab is being deposited with the ATCC as described in Example

10 7 along with Fab 12 and Fab 13.

There are a number of conclusions arising from the data shown in the Figures 6 and 9. It is apparent that HIV-1 can be neutralized without virion aggregation or cross-linking of gp120 molecules on the

15 virion surface since monovalent Fab fragments are effective. To further confirm this finding, a Fab fragment was produced by papain digestion of a known neutralizing human monoclonal antibody. As shown in Figure 6, the Fab fragment was approximately equally

20 effective as the whole IgG in neutralization of the MN strain of HIV-1. This is consistent with results on Fabs prepared from two mouse monoclonal antibodies to the V3 loop. An F(ab')$_2$ fragment of a mouse monoclonal antibody was somewhat less effective than

25 the parent IgG in neutralization of the MN strain. Interestingly, the fragments from these control antibodies were relatively poor in neutralizing the IIIB strain of HIV-1. The results also show that there appears to be a difference between the two

30 assays employed since Fab 13 was consistently effective in one assay but not the other. The principal variables were the incubation time of the virus and antibody prior to infection (2 hours for the p24 assay and 0.5 hours for the syncytial assay), the

35 amount of virus used for infection, the cells used to

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propagate virus (human PBMCs for the former and H9
cells for the latter) and the cells infected (human
PBMCs for the former and CEM.SS cells for the latter).
Of these, there is a strong possibility that the MN

5   virus used in the two assays, having been passaged
through different cells, is critically different.

       d.   Results of the Neutralization Assays for
             gp41

10   The gp41-reactive Fabs exhibited specificity
to the conformation epitope of gp41 including amino
acid residues in positions 565-585 and 644-663. The
five selected gp41-specific Fabs were designated DL 41
19, DO 41 11, GL 41 1, MT 41 12 and SS 41 8.

15   Neutralization assays were performed as described
above for the gp120-reactive Fabs. In the plaque
assays, the data shown is the concentration of Fab in
micrograms/milliliter required to achieve 50% of
neutralization. The data for the other two

20   neutralization assays is also expressed in
micrograms/milliliter of Fab required to neutralize
infection as defined in the description of the p24 and
syncytial assays above. The results of the three
neutralization assays, plaque, syncytial and p24, are

25   presented in Table 4. The MN and IIIB HIV strains
were used as indicated in Table 4 for the assays. The
abbreviation "ND" stands for not determined when
indicated in the table.

30   Table 4
                        Assay/Strain

		Plaque		Syncytial		P24	
	Fab	MN	IIIB	IIIB		MN	IIIB
35	DL 41 19	<4	<40	1.4		ND	ND

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DO 41 11	<40	7.1	2.3	0.9	ND
GL 41 1	<4	<4	1.7	ND	3.5
MT 41 12	<40	<40	5.5	4.5	4.5
SS 41 8	<4	<4	2.2	ND	7.1

As shown in Table 4, all five Fabs were effective at neutralizing both MN and IIIB strains of HIV in either plaque, syncytial or p24 assays. Fabs DL 41 19 and DO 41 11 exhibited strain specificity in the plaque assay where the former was ten-fold more effective at inhibiting plaque formation with the MN strain than with the IIIB strain. The opposite specificity was seen with the DO 41 11 Fab. However, both Fabs exhibited comparable neutralization as measured by the syncytial assay. Two Fabs, GL 41 1 and SS 41 8, were equally effective at inhibiting plaque formation with either MN or IIIB strains. The Fab MT 41 12 was similarly not strain-specific although neutralization required 10 fold more antibody. No strain specificity was evident when Fab MT 41 12 was used in p24 assays where the same amount of antibody was equally effective. All five antibodies were neutralized IIIB as measured in the syncytial assay.

Thus, the five gp41-specific Fabs neutralized HIV-1 MN and IIIB in at least two of the three assays used for measuring neutralizing activity. Moreover, strain specificity was prevalent in two of the five assays as measured by the plaque assay. Based on these differential neutralization characteristics, the gp41-specific Fabs provide useful therapeutic reagents for neutralizing HIV-1.

4.   Nucleic Acid Sequence Analysis Comparison Between HIV-1 Specific Monoclonal Antibody Fabs and the

Corresponding Derived Amino Acid Residue Sequence

To explore the relationship between neutralizing and weakly or non-neutralizing Fabs, the variable domains of 32 clones expressing human anti-gp120 Fabs,
5   prepared in Example 2 including the 20 listed in Figure 6 for which neutralizing activity was assessed, were sequenced. In addition, the five gp41-specific Fabs were also sequenced.

Nucleic acid sequencing was performed on
10   double-stranded DNA using Sequenase 1.0 (USB, Cleveland, OH) and the appropriate primers hybridizing to sequences in the Cg1 domain (SEQGb : 5' GTCGTTGACCAGGCAGCCCAG 3' SEQ ID NO 49) or the Ck domain (SEQKb : 5' ATAGAAGTTGTTCAGCAGGCA 3' SEQ ID NO
15   50). Alternatively sequencing employed single stranded DNA and the T3 primer (5' ATTAACCCTCACTAAAG 3', SEQ ID NO 51) or one hybridizing to a sequence in the Ck domain (KEF : 5' GAATTCTAAACTAGCTAGTTCG 3' SEQ ID NO 52).

20   The amino acid residue sequences of the variable heavy and light chains derived from the nucleic acid sequences of the 32 gp120-specific clones are shown respectively in Figures 10 and 11. Groupings are made on the basis of similarities in heavy chain sequences.
25   Dots indicate identity with the first sequence in each section. The SEQ ID NOs are listed to the right of the corresponding derived heavy and light chain (V_H from SEQ ID NO 53-81 and V_L from SEQ ID NO 82-113) amino acid residue sequences in the Figures
30   themselves.

Alignment of derived sequences with one another and with the Genbank database made use of the MacVector suite of programs. For analysis of heavy chain CDR3 sequences as described by Sanz, J.
35   Immunol., 147:1720-1729 (1991), the most 5' nucleotide

was considered to be the first nucleotide after codon
95 of the H chain variable region according to Kabat
et al, Sequences of Proteins of Immunological
Interest, US Dept. of Health and Human Services,

5   Washington, DC (1991). The most 3' nucleotide was
assigned to the last unidentified nucleotide before
the sequence matched with the published germline JH
genes. The CDR3 sequences were analyzed using the
DNASTAR software. Sequence comparisons were performed

10   with both the ALIGN and COMPARE programs in order to
determine the germline D gene which provided the best
homology throughout. In a second step, the SEQCOMP
program was used to find sequence identity of at least
six nucleotides with either the coding strand or the

15   reverse complement of germline D genes.

The heavy and light chain sequences of the gp41-
specific Fabs are shown in Figures 18 and 19,
respectively.   The amino acid residue sequence of the
CDR3 heavy chain exhibits the most variation between

20   the Fabs than any other region of the variable domain.

       a.   Organization of Antibodies into Groups
             According to Heavy Chain Sequence

V_H and V_L domains of 32 gp120 clones were

25   sequenced and the V_H domains compared using MacVector
software. This analysis immediately established that
a number of the clones, including those selected by
panning against different antigens, are closely
related to one another. The exception to this is the

30   Fabs selected by panning against the V3 loop peptide
which are not related to the Fabs selected by panning
against the gp120/160 antigens. Figure 10 shows that
the V_H sequences derived from gp120/160 panning can be
organized into 7 groups. The broad features apparent

35   from a comparison of amino acid sequences are

discussed herein.

The relatedness of sequences within a group varies considerably. For instance, in the group beginning with clone number b8 the amino acid

5   sequences are very similar. Six clones were identical and the remainder showed a maximum of 5 differences from the predominant sequence (the EQ difference due to the 5' primer excluded). Only one clone showed a single difference in the CDR3 region. The average

10   discrepancy over all the sequences in this group from the predominant sequence is 1.1 amino acid residues/ variable domain. This amount corresponds to the order of magnitude of discrepancies which could arise from the PCR. Sequencing of constant domains indicated a

15   PCR error frequency of about 1 base change per domain.

In contrast, in the group headed by clone b3, no two clones were absolutely identical. The average difference from the consensus group sequence is 3.3 residues per sequence and determination for the CDR3

20   alone is 1.3. Therefore, it seems likely that the heavy chains in this group are somatic variants of one another.

The group headed by clone 1 presents a third pattern. Clones b1 and b14 are identical as are

25   clones b2 and B2. However, 23 amino acid differences exist between the two sets of clones. Clones b24 and B30 are approximately equally well differentiated (13-25 differences) from either of these two sets of clones or one another. Still the CDR3 regions are

30   very similar. A number of explanations can be suggested for this pattern: 1) all clones in this group originate from the same germline gene which has undergone extensive somatic mutation, 2) cross-over events have occurred to essentially recombine

35   different germline genes with the same DJ combination,

3) a "convergent evolution" process has led to the
selection of different germline genes associated with
the same DJ combination.

     b.   <u>Sequences of the V_L Domains from the gp120
Binders</u>

The V_L sequences of the Fabs were organized
into the groups defined in Figure 10 are shown in
Figure 11. Immediately apparent was the extensive
chain promiscuity as evidenced by the pairing of
different light chains with the same or a very similar
heavy chain with retention of antigen binding
capability and indeed, for the most part, antigen
affinity as compared with Figure 10. This promiscuity
can be explored further by reference to the groups
considered above.

The clone b8 group, in which the heavy chain
members were identical or very similar, also produced
4 light chains which are identical or very similar
(less than 3 amino acid differences). Therefore a
predominant heavy-light chain combination can be
described for this group. One member (clone b8) had
the same or very closely related V_L gene but appeared
to use a different Jk gene. Two other members (clones
B8 and b18) were more distantly related to the major
sequence (7-12 differences). Two further clones (b13
and B26) used a Vk gene from a different family, Vk3
compared to Vk1, and therefore were unrelated to the
major sequence.

The clone b3 group, suggested to contain somatic
variants of a single heavy chain, showed considerable
light chain diversity with no two members being
closely related to one another. Vk3-Jk2 combinations
predominated but Vk3-Jk3 and Vk1-Jk3 combinations also
occurred.

On the other hand, in the clone b1 group evidence
existed for the heavy chains being more choosy about
their light chain partner. Thus, closely related
heavy chains appeared to be paired with related light
5   chains. The identical heavy chain pairs (b1 and b14;
b2 and B2) had very similar light chains (2 and 4
amino acid differences respectively) whereas the
distinct heavy chains (b24 and B30) had distinct light
chains which were unrelated to one another or the
10   other group members. The clone 4 group provides
another example of this phenomenon in that 4 closely
related heavy chains were paired with 3 closely
related light chains (a predominant heavy-light chain
combination), except for the clone b7 light chain that
15   was distinct.

In summary, the heavy chain (V_H) sequences was
organized into 7 groups where each member of a group
has an identical or very similar CDR3 region with a
limited number of differences elsewhere. When the
20   light chains (V_L) were constrained into the groupings
defined by their heavy chain partners, considerable
light chain sequence variation was observed. This
phenomenon of chain promiscuity has been observed
previously and can be appreciated by reference to
25   Figure 11. Marked neutralizing ability was confined
to two groups of sequences. The first group consisted
of Fabs 4, 7, 12 and 21 which have very similar heavy
and light chains. The second group consisted of Fabs
13, 8, 18, 22 and 27. Only Fab 13 showed marked
30   neutralizing ability, although the others showed some
weaker activity. Interestingly in this group Fab 13
did have a light chain distinct from the other members
of the group.

35   5.   Shuffling of the Heavy and Light Chain of a

Single Clone Against the Library

To further explore possible functional heavy-light chain combinations, the heavy chain of clone b12 (also referred to as Fab 12 for the corresponding soluble Fab preparation) shown in Figure 10 was recombined with the original light chain library prepared in Example 2 to construct a new library H12-LCn. In addition, the b12 light chain was recombined with the original heavy chain library to construct a library Hn-L12. These two libraries were taken through 3 rounds of panning against gp120 (IIIB) as described in Example 2b5). The Fabs expressed from the resultant immunoreactant clones were analyzed as described in Example 3 above. Clone b12 was chosen as this Fab neutralized HIV-1 _in vitro_ as shown in Example 3.

To accomplish the preparation of a shuffled library from the Fd gene of clone b12 with the original light chain library, the b12 heavy chain was first subcloned into a tetanus toxoid binding clone expressed in pComb2-3. The light chain library was then cloned into this construction to give a library of 1×10^7 members. The subcloning step was used to avoid contamination with and over-representation of the original light chain. A similar procedure was adopted for shuffling of heavy chains against the light chain from clone b12 to give a library of 3×10^6 members. Cloning and panning procedures were carried out as described above for the original library.

Eleven light chains which recombined with the b12 heavy chain and bound gp120 by panning were randomly chosen for subsequent competition ELISA and sequence analysis. The apparent affinities of these shuffled combinations were similar with an IC_{50} of

approximately 10^{-8} to 10^{-9} M. The sequences were
organized where a set of 3 were very similar to the
original b12 light chain and the other 8 showing many
differences from the original with some sub-grouping

5   possible.

The sequences of the light chains which bound to
the b12 heavy chain clone are shown in Figure 12. The
sequences are compared to the sequence for the
original light chain from clone b12. The light chains

10   are identified by numbers which do not correspond to
the original light chain clones; the assigned numbers
of the newly selected clones having new light chains
are thus arbitrary. The sequences of these light
chains are also listed in the Sequence Listing from

15   SEQ ID NO 114 to 122. Some light chain sequences are
identical. In addition to immunoreactivity with
gp120, the new Fabs isolated from these shuffled
clones were tested in the syncytia assay for
neutralization of HIV-1 infection as described in

20   Example 3. Four shuffled monoclonal Fab antibodies,
each having the heavy chain from clone b12, a known
HIV-1 neutralizing clone, and new light chains
designated L28, L25, L26 and L22, all exhibited
approximately 60% neutralization in a syncytia assay

25   with 0.4 μg/ml purified Fab. This effect was
equivalent to that obtained with the original clone
b12 heavy and light chain pair. Maximum
neutralization of approximately 80% was obtained with
the H12/L28 and H12/L25 Fabs at 0.7 μg/ml which was

30   equivalent to that seen with the original clone b12
heavy and light pair. The neutralization resulting
from the H12/L22 and H12/L26 Fabs plateaued at 60%
with Fab concentrations of 0.4 μg/ml up to 1.0 μg/ml.
Thus, in addition to the gp120 immunoreactive and HIV

35   neutralizing Fabs obtained in the original library

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prepared as described in Example 2, by shuffling a
known neutralizing heavy chain with a library of light
chains, new HIV-1 neutralizing Fab monoclonal
antibodies have been obtained.

5   Ten heavy chains which recombined with the b12
light chain were also randomly chosen. One was very
similar to the original b12 heavy chain but the others
have many differences. Nevertheless, the V-D and D-J
junctions were essentially identical indicating the

10   clones had probably arisen from the same rearranged
B-cell clone by somatic modification. Competition
ELISA failed to reveal any clear difference in
affinity between the variants selected from those
originally analyzed.

15   The sequences of the heavy chains which bound to
the b12 light chain clone are shown in Figure 13. The
sequences are compared to the sequence for the
original heavy chain from clone b12. The heavy chains
are identified by numbers which do not correspond to

20   the original light chain clones; the assigned numbers
of the newly selected clones having new heavy chains
are thus arbitrary. The sequences of these light
chains are also listed in the Sequence Listing from
SEQ ID NO 123 to 132. Some light chain sequences are

25   identical. In addition to immunoreactivity with
gp120, the new clones were tested in the syncytia
assay for neutralization of HIV-1 infection as
described in Example 3. Two shuffled monoclonal Fab
antibodies, each having the light chain from clone

30   b12, a known HIV-1 neutralizing clone, and new heavy
chains designated H2 and H14, exhibited approximately
40% neutralization in a syncytia assay with 1.0 and
0.5 μg/ml purified Fab, respectively. This effect was
equivalent to that obtained with the original clone

35   b12 heavy and light chain pair at a concentration of 2

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µg/ml. Maximum neutralization of approximately 50%
was obtained with the Fab having the new H14 chain at
1.0 µg/ml compared to 80% neutralization with 0.7
µg/ml with the original clone b12 heavy and light

5   pair. Thus, in addition to the gp120 immunoreactive
and HIV neutralizing Fabs obtained in the original
library prepared as described in Example 2, by
shuffling a known neutralizing light chain with a
library of heavy chains, new HIV-1 neutralizing Fab

10   monoclonal antibodies have been obtained.

Thus, this shuffling process revealed many more
heavy and light chain partners that bound to gp120
that were equal in affinity to those obtained from the
original library prepared in Example 2. With this

15   approach, additional HIV-1 neutralizing antibodies can
easily be obtained over those present in an original
library. The complexity of the clones arising from
the heavy chain shuffling also suggests that this
approach may be used to map the course of somatic

20   diversification.

Combinatorial libraries randomly recombine heavy
and light chains so to what extent antibodies derived
from such libraries represent those produced in a
response in vivo can be determined. In principle, a

25   heavy-light chain combination binding antigen could
arise fortuitously, i.e., neither chain is involved in
binding antigen in vivo but the combination does bind
antigen in vitro.

The available data suggests, however, that heavy

30   chains, from immune libraries, involved in binding
antigen tightly in vitro arise from antigen-specific
clones in vivo. First, studies have generally failed
to identify high-affinity binders in non-immunized IgG
libraries. See, Persson et al. Proc. Natl. Acad.

35   Sci., USA, 88:2432-2436 (1991) and Marks et al. Eur.

J. Immunol., 21:985-991 (1991).

Further, as described above, gp120 binders were
not observed in panning a bone marrow IgG library from
an HIV seronegative donor against gp120. Second,
5   heavy chains associated with binders from immunized
libraries were typically at relatively high frequency
in the library indicating they were strongly
represented in the mRNA isolated from immunized
animals. See, Caton et al., Proc. Natl. Acad. Sci.,
10   USA, 87:6450-6454 (1990) and Persson et al., supra.
Third, heavy chains from immunized libraries appeared
to dictate specificity when recombined with various
unrelated light chains as described in Example 6.
Fourth, the isolation of intraclonal heavy chain
15   variants as here indicated that an active antibody
response was cloned. Thus, the shuffling of a known
heavy chain with a light chain binder and vice versa
is preferred for use in this invention as new
neutralizing Fabs can be obtained beyond those
20   generated in vivo.

Heavy chain promiscuity, i.e., the ability of a
heavy chain to pair with different light chains with
retention of antigen affinity, presents serious
problems for identifying in vivo light chain partners.
25   This applies not only to the strict definition of
partners as having arisen from the same B-cell but
also to one which would encompass somatic variants of
either partner. The existence of predominant
heavy-light chain combinations, particularly involving
30   intraclonal light chain variants, suggests that the
light chains concerned are well represented in the
library and probably are associated with antigen
binding in vivo. However, promiscuity means that,
although some combinations probably do occur in vivo,
35   one cannot be certain that one is not shuffling immune

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partner chains in the recombination. For instance,
the occurrence of a virtually identical light chain
(b6, B20) in 2 out of 33 clones suggests that it is
probably over-represented in the library consistent
5   with an in vivo involvement in antigen-stimulated
clones. However, there is no way of knowing whether
the in vivo partner of the light chain is the b6 or
B20 heavy chain or indeed another heavy chain arising
from a stimulated clone.

10   The light chains arising from the combinatorial
library may not be those employed in vivo.
Nevertheless it is interesting to note that some heavy
chains appear relatively choosy about light chain
partner whereas others appear almost indifferent.
15   This observation needs to be tempered by the finding
that apparently choosy heavy chains from this analysis
will accept diverse light chains with maintenance of
antigen binding in a binary plasmid system where
pairings are forced as shown below in Example 6 rather
20   than selected in a competitive situation.

         Two reports compare heavy-light chain
combinations arising from combinatorial libraries and
hybridomas in immunized mice. The library approach
begins with mRNA and is therefore probably reflecting
25   plasma cell populations. In contrast, hybridomas are
thought to reflect activated but not terminally
differentiated B cell populations and EBV
transformation to reflect resting B cell populations.

         Whatever the arguments about light chain
30   authenticity, the heavy chains of Figure 10 present
many features of interest. The most frequently used
heavy chain is of the clone b8 type. It could be
argued that this usage simply represents bias in PCR
amplification. However, the occurrence of
35   approximately equal numbers of clones in this group

amplified by VH1a and VH3a primers argues against this notion. Furthermore, the existence of intraclonal variants in some groups indicates that one is at least sampling different genes from the initial library.

5   The antibodies cloned here do bear qualitative relationship with the polyclonal antibodies present in the serum of the asymptomatic donor. The titer of anti-gp120 (IIIB) antibodies was approximately 1:3000, with greater than 50% of the reactivity being

10   inhibited by CD4 or a cocktail of Fabs from clones 12, 13 and 14. The titer of anti-gp120 (SF2) antibodies was approximately 1:800. Further, the titer of serum against the short constrained V3 loop peptide was 1:500 and against the full length MN V3 loop peptide

15   was only 1:300. The importance of "anti-CD4 site antibodies" seems general in donors with longer term HIV infection in that the cocktail of Fabs 12, 13 and 14 was able to inhibit binding of a large fraction of serum antibody reactivity with gp120 (IIIB) in 26 of

20   28 donors tested.

The ability of Fabs to neutralize viruses has been a controversial area. One of the problems has been that Fabs are classically generated by papain digestion of IgG. If the Fab, as is often the case,

25   shows reduced activity relative to the parent IgG then it may be difficult to rule out IgG contamination in the Fab preparation. Recombinant Fabs, however, as shown herein definitively neutralize virus.

The mechanism of neutralization of HIV-1 appears

30   to neither require virion aggregation nor gp120 cross-linking. In addition, there is no correlation with blocking of the CD4-gp120 interaction to neutralization. The existence of the cloned neutralizing Fabs of this invention should allow the

35   molecular features that confer neutralizing potential

to be explored. For instance, in the case of the
group of clones containing Fab 13, the unique
character of the light chain of that neutralizing
clone suggests that chain shuffling experiments in
5   which the 13 light chain was recombined with the other
heavy chains in that group, might be revealing. Heavy
chains paired with two dissimilar light chains have
been shown to retain antigen affinity but exhibit
altered fine specificity as shown in Example 6.

10   The observation here of a large number of Fabs
with only a limited number being strongly neutralizing
may have important consequences. If the pattern is
repeated for whole antibodies then it would seem that
much of the gp120 structure may be in a sense a
15   "decoy", i.e., the immune system may invest
considerable effort in producing antibodies of high
affinity but limited anti-viral function. To
exacerbate the situation the ineffective antibodies
may bind to gp120 and inhibit the binding of strongly
20   neutralizing antibodies. This has obvious
consequences for vaccination which should be primarily
designed to elicit neutralizing antibodies of this
invention.

25   6.   Shuffling of Selected Heavy and Light Chain DNA
            Sequences of a Combinatorial Library in a Binary
            Plasmid System
            A binary system of replicon-compatible plasmids
has been developed to test the potential for
30   promiscuous recombination of heavy and light chains
within sets of human Fab fragments isolated from
combinatorial antibody libraries. The efficiency of
the system is demonstrated for the combinatorial
library of this invention derived from the bone marrow
35   library of an asymptomatic HIV donor.

a. Construction of the Binary Plasmid System

The binary plasmids pTAC01H and pTC01 for
use in this invention contain the pelB leader region
and multiple cloning sites from Lambda Hc2 and Lambda
5   Lc3, respectively, and the set of replicon-compatible
expression vectors pFL281 and pFL261. Both pFL281 and
pFL261 have been described by Larimer et al., Prot.
Eng., 3:227-231 (1990), the disclosure of which is
hereby incorporated by reference. The nucleotide
10   sequences of pFL261 and pFL281 are in the EMBL,
GenBank and DDBJ Nucleotide Sequence Databases under
the accession numbers M29363 and M68946. The plasmid
pFL281 is based on the plasmid pFL260 also described
by Larimer et al., supra, and having the accession
15   number M29362. The only distinction between the
plasmids pFL260 and pFl281 is that pFL281 lacks a 60
bp sequence of pFL260 between the Eag I site and the
Xma III site resulting in the loss of one of the two
BamH I sites. This deletion is necessary to allow for
20   cloning of the BamH I Hc2 fragment into the expression
vector as described herein.

The replicon-compatible expression vectors share
three common elements: (i) the f1 single-stranded DNA
page intergenic IG regions; (ii) the tightly regulated
25   tac promoter and lac operator; and (iii) an rbs-ATG
region with specific cloning sites. The plasmid
vectors differ in their antibiotic resistance markers
and plasmid replicons: pFL261 carries a gene encoding
chloramphenicol acetyltransferase (cat), conferring
30   chloramphenicol resistance,a nd the p15A replicon;
pFL281 carries a gene encoding beta-lactamase (bla),
conferring ampicillin resistance, and the ColE1
replicon (ori) from pMB1. The p15A and ColE1
replicons permit the coincident maintenance of both
35   plasmids in the same E. coli host.

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The Hc2 and Lc2 vectors prepared in Examples 1a2) and 1a3), respectively, were converted into the plasmid form using standard methods familiar to one of ordinary skill in the art and as described by Sambrook

5   et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) and subsequently digested with Xho I-Spe I (pHc2) and Sac I-Xba I for (pLc2). The synthetic linkers for insertion into the digested pHc2 and Lc2

10   plasmids were prepared by American Synthesis. The linkers were inserted to increase the distance between cloning sites so as to increase the effectiveness of the digestions. The 5' and 3' linkers for preparing the double-stranded linker insert into pHc2 were 5'

15   TCGAGGGTCGGTCGGTCTCTAGACGGTCGGTCGGTCA 3' (SEQ ID NO 133) and 5' CTAGTGACCGACCGACCGTCTAGAGACCGACCGACCC 3' (SEQ ID NO 134), respectively. The 5' and 3' linkers for preparing the double-stranded linker insert into pLc2 were 5' CGGTCGGTCGGTCCTCGAGGGTCGGTCGGTCT 3' (SEQ

20   ID-NO 135) and 5' CTAGAGACCGACCGACCCTCGAGGACCGACCGACCGAGCT 3' (SEQ ID NO 136), respectively. The pairs of linker oligonucleotides were separately ligated to their respective digested, calf intestinal phosphatase-

25   treated vectors.

Subsequently, the multiple cloning sites of pHc2 and pLc2 were transferred into the expression vectors, pFL281 and pFL261, respectively. To accomplish this process, the multiple cloning regions of both Lc2 and

30   Hc2 were separately amplified by PCR as described by Gram et al., Proc. Natl. Acad. Sci., USA, 89:3576-3580 (1992) and as described in Example 2b using Vent Polymerase (New England Biolabs) according to the manufacturer's recommendations. The forward primer,

35   5' CAAGGAGACAGGATCCATGAAATAC 3' (SEQ ID NO 137) was

designed to provide a flush fusion of the pelB leader
sequence to the ribosome binding sites of the cloning
vectors pFL261 and pFL281 via its internal BamH I site
indicated by the underlined nucleotides. The reverse

5   primer 5' AGGGCGAATTGGATCCCGGGCCCCC 3' (SEQ ID NO 138)
was designed to anneal downstream of the region of
interest in the parent vector of pHc2/pLc2 and create
a second BamH I site. The resultant Hc2 and Lc2 PCR
amplification products were then digested with BamH I

10.   to provide for BamH I overhangs for subsequent
ligation into BamH I linearized pFL281 and pFL261
vectors, respectively. The resulting light chain
vector containing the Lc2 insert, designated pTC01,
was used in this form, whereas the heavy chain vector

15   was further modified with a histidine tail to allow
purification of Fab fragments by immobilized metal
affinity chromatography as described by Skerra et al.,
Bio/Technology, 9:273-278 (1991). For this purpose,
the synthetic linker oligonucleotides, respectively

20   the 5' and 3' linkers, 5'
CTAGTCATCATCATCATCATTAAGCTAGC 3' (SEQ ID NO 139) and
5' CTAGGCTAGCTTAATGATGATGATGATGA '3 (SEQ ID NO 140)
was inserted into the Spe I site, in effect removing
the decapeptide tag sequence to generate the heavy

25   chain vector designated as pTAC01H. The expression of
Fab fragment in all subsequent cloning experiments was
suppressed by adding 1% (w/v) glucose to all media and
plates.

30   b.   Construction of Expression Plasmids
For expression of the light chain variable
domain, pTC01 prepared above was first digested with
Sac I and Xba I; individual light chain inserts were
then obtained by separately digesting 22 of the

35   pComb2-3 plasmids prepared and screened as described

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in Example 2 and listed in Figure 7 that bind to gp120
with the same combination of enzymes and isolating the
0.7 kb fragment using low melting point agarose gel
electrophoresis followed by b-agarose digestion. For
5   the chain-shuffling experiments, the following
representative members of each of the seven groups
shown in Figure 7 were chosen: b11; b6; b4-b12-b7-b21;
b3; s8; b1-b14-b24; b13-b22-B26-b8-b18-b27-B8-B35-s4;
and one loop peptide-binding clone, p35. The
10   different groups are indicated by semicolon
separations while members of the same group are
dashed. The resultant isolated light chains were
separately ligated into PTCO1 overnight at 16°C under
standard conditions using a 5:1 molar insert-to-vector
15   ratio to form 21 light chain pTCO1 expression vectors.

For expression of the heavy chain variable
domain, pTAC01H prepared above was first digested with
Xho I and Spe I; heavy chain inserts were then
obtained by separately PCR amplification reactions of
20   the 20 pComb2-3 plasmids from which light chain
inserts were obtained. PCR was used to isolate the
heavy chain inserts instead of restriction digestion
in order to obtain heavy chain without the cpIII gene
anchor sequence in the vector. For the PCR reaction,
25   the respective 5' and 3' primers, 5'
CAGGTGCAGCTCGAGCAGTCTGGG 3' (VH1a) (SEQ ID NO 42) and
5' GCATGTACTAGTTTTGTCACAAGATTTGGG 3' (CG1z) (SEQ ID NO
44) were used to amplify the region corresponding to
the heavy chain as described in Examples 2a1) and
30   2a2). The resultant PCR products were purified by
low-melting point electrophoresis, digested with Xho I
and Spe I, re-purified, and separately ligated to the
similarly prepared heavy chain pTAC01H vector using a
1:2 molar vector-to-insert ratio to form 21 heavy
35   chain pTACO1H expression vectors.

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c. Co-transformation of Binary Plasmids

CaCl$_2$-competent XL1-Blue cells (Stratagene;
recA1, endA1, gyrA96, thi, hsdR17, supE44, relA1, lac,
{F' proAB, lacI^q, ZDM15, Tn10(tet^R)}) were prepared
5   and transformed with approximately 0.5 µg purified DNA
of each plasmid in directed crosses of each of the 20
light chain vectors with each of the 20 heavy chain
vectors. The presence of both plasmids and the
episome was selected for by plating transformants on
10   triple-antibiotic agar plates (100 µg/ml
carbenicillin, 30 µg/ml chloramphenicol, 10 µg/ml
tetracycline, 32 g/l LB agar) containing 1% glucose.

A binary plasmid system consisting of two
replicon-compatible plasmids was constructed as shown
15   in 14. The pTAC01H heavy chain vector schematic is
shown in Figure 14A and the pTCO1 light chain vector
schematic is shown in Figure 14B. Both expression
vectors feature similar cloning sites including pel B
leader sequences fused to the ribosome binding sites
20   and the tac promoters via BamH I sites as shown in
Figure 15. The nucleotide sequences of the multiple
cloning sites along with the tac promoter, ribosome
binding sites (rbs) and the underlined relevant
restriction sites for the light chain vector, pTCO1,
25   and heavy chain vector, pTAC01H, are respectively
shown in Figure 15A and Figure 15B. The sequences are
also listed in the Sequence Listing as described in
the Brief Description of the Drawings. The heavy
chain vector pTAC01H also contains a (His)$_5$-tail to
30   allow purification of the recombinant Fab fragments by
immobilized metal affinity chromatography. The
presence of both plasmids in the same bacterial cell
is selected for by the presence of both antibiotics in
the media. Expression is partially suppressed during
35   growth by addition of glucose and induced by the

addition of IPTG at room temperature. Under these
conditions, both plasmids are stable within the cell
and support expression of the Fab fragment as assayed
by ELISA using goat anti-human kappa and goat
5   anti-human IgG1 antibodies.

       d.   Preparation of Recombinant Fab Fragments
             Bacterial cultures for determination of
antigen-binding activity were grown in 96 well-tissue
10   culture plates (Costar #3596). 250 μl Superbroth [SB
had the following ingredients per liter: 10 g 3-(N-
morpholino) propanesulfonic acid, 30 g tryptone, 20 g
yeast extract at pH 7.0 at 25°C) containing 30 μg/ml
chloramphenicol, 100 μg/ml carbenicillin, and 1%
15   (w/v)] glucose were admixed per well and inoculated
with a single double-transformant prepared in Example
6c above. The inoculated plates were then maintained
with moderate shaking (200 rpm) on a horizontal shaker
for 7-9 hours at 37°C, until the A_{550} was approximately
20   1-1.5. The cells were collected by centrifugation of
the microtiter plate (1,500 X g for 30 minutes at
4°C), the supernatants were discarded, and the cells
were resuspended and induced overnight at room
temperature in fresh media containing 1 mM IPTG, but
25   no glucose. Cells were harvested by centrifugation,
resuspended in 175 μl PBS (10 mM sodium phosphate, 160
mM NaCl at pH 7.4 at 25°C) containing 34 μg/ml
phenylmethylsulfonyl fluoride (PMSF) and 1.5% (w/v)
streptomycin sulfate, and lysed by 3 freeze-thaw
30   cycles between -80°C and 37°C. The resultant crude
extracts were partially cleared by centrifugation as
above before analysis by antigen-binding ELISA.

       e.   Assay and Determination of Relative
35   Affinities

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Relative affinities were determined as described in Example 2b6) after coating wells with 0.1 μg of antigen. The selected antigens included tetanus toxoid and recombinant gp120 (strain IIIB) and gp120 (strain SF2). For each antigen, a negative control extract of XL1-Blue cells co-transformed with pTC01 and pTAC01H was tested to determine whether other components in E. coli had any affinity for the antigens in the assay. Each extract was assayed for BSA-binding activity and BSA-positive clones were considered negative. All possible single-transformants expressing one chain only were prepared as described for the double-transformants and were found to have no affinity for any of the antigens used. Because of the nature of the assay, whether this was due to a lack of binding by the individual chains itself or due to a lack of expression or folding could not be determined.

f.   Results of Direct Crosses of Heavy and Light Chains within a Set of gp120/gp160 Binding Antibodies

The Fab fragments derived from the bone marrow of the same asymptomatic HIV donor but panned against gp120 (IIIB), gp160 (IIIB), and gp120 (SF2), were assigned to one of seven groups based on the amino acid sequences of the CDR3 of their heavy chains as described in Example 4. From the same library, antibodies to the constrained hypervariable v3-loop-like peptide JSISIGPGRAFYTGZC (SEQ ID NO 141) were isolated. For the chain-shuffling experiments, the following representative members of each of the seven groups shown in Figure 7 were chosen: b11; b6; b4-b12-b7-b21; b3; s8; b1-b14-b24; b13-b22-B26-b8-b18-b27-B8-B35-s4; and one loop peptide-binding clone, p35.

Clones b4, b7, b12, and b21 showed neutralization
activity against HIV when monitoring inhibition of
infection by syncytia formation and clones b13, b12,
and b4 when monitoring p24 production as shown in
Example 3. Light and heavy chains were cloned from
the original constructs and cotransformed in all
possible binary combinations into XL1-Blue cells as
described above.

The results of the complete cross are shown in
Figure 16. As is to be expected, identical chains
derived from different Fab fragments had similar
binding properties e.g., b18HC, b27HC, B8HC, B35HC,
s4HC. The crosses of the original heavy chains with
the original light chains in each case clearly
recapitulated binding activity. Minor differences
existed between some heavy chains with identical
variable domain sequences, e.g., b4 and b12 (constant
domains were not sequenced for any of the constructs).
The exception is b8HC, which was identical in its
variable domain to b18HC, b27HC, B8HC, B35HC, s4HC,
yet shows more cross reactivity. Presumably, this is
due to differences in expression levels in the cell or
differences in the constant domain sequences. Clear
differences existed between heavy chains in their
tendency to accept different light chains and still
bind antigen, but even the least promiscuous heavy
chain in the set panned against gp120 (IIIB), b1HC,
still did so in 43% of its crosses. On the other side
of the spectrum, 5 heavy chains, b11HC, b6HC, b12HC,
b7HC, and b8HC, crossed productively with all light
chains in this set. For the heavy chain crosses
examined in detail (all of s4HC, B35HC, B26HC; most of
b12HC, b12HC), no significant differences in apparent
binding affinity were found between Fab fragments
using the same heavy chain but different light chains

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as shown in Figure 17 where the IC_{50} from competition with soluble gp120 (IIIB) was approximately 10^{-8} M.

Within the original seven groups that were established according to the sequence of the CDR3 of the heavy chains and that are indicated by horizontal and vertical lines in Figure 16, complete promiscuity was present, i.e., heavy and light chains within these CDR3-determined groups were completely promiscuous with each other. However, there was a lack of promiscuity between other groups, e.g., between b1HC-b24HC and b13LC-s4LC. In the analysis of these sequence-based groups, the protein antigen against which the phage display library was panned was not a critical factor. The exception to this case was the cross of p35HC with all light chains; the only cross that bound either to gp120 (SF2 strain) or the original antigen, the loop peptide, was the cross containing the original heavy and light chains.

Unlike the heavy chains, no light chains crossed productively with all heavy chains nor were any distinguishable from the other light chains by unusually low promiscuity.

In the neutralization assays performed as described in Example 3, the directed cross resulting from the pairing of the heavy chain from clone b12 with the light chain from clone b21, was effective at neutralizing HIV-1.

g.   Interantigenic Crosses of Heavy and Light Chains

To determine whether conclusions derived from the crosses between high affinity Fab fragments originating from the same library can be extended to unrelated libraries, a non-related gamma1k-Fab fragment (P3-13) specific for tetanus toxoid from a

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different donor was chosen for a new set of crosses
[clone 3 in Persson et al., Proc. Natl. Acad. Sci.,
USA, 88:2432-2436 (1991)]. Extracts were probed with
tetanus toxoid or with gp120 (IIIB). The data confirm
5   the results from the gp120 cross experiment in that
the binding activity towards the antigen was
determined by the heavy chain. The heavy chain of
clone P3-13 paired with the light chains b4, b12, b21,
and b14 to yield an Fab fragment with an affinity
10   towards tetanus toxoid; the light chain of P3-13
paired with the heavy chains of b3, b6, b11, and b14
to yield an Fab fragment with an affinity towards
gp120 (IIIB). None of the light chains originating
from the gp120 binders was able to confer gp120
15   specificity in combination with the P3-13 heavy chain.
        Similarly, the P3-13 light chain was unable to
generate tetanus toxoid specificity in combination
with any of the heavy chains originating from the
gp120 binders, confirming the dominance of the heavy
20   chain in the antibody-antigen interaction.
Interestingly, all three light chains that showed a
strong signal against tetanus toxoid (b4, b12, b21)
were members of the same group when sorted by the
CDR3's of their original heavy chains. As might be
25   expected from crosses between unrelated libraries, not
only was there a lower degree of promiscuity, i.e.,
chains paired productively with far fewer
complementary chains, but the range of apparent
affinity constants determined by competition ELISA was
30   much broader ($6.3 \times 10^6 - 6.3 \times 10^{-8}$ M). The
replacement of the original P3-13 light chain in the
P3-13 Fab fragment with another light chain lowered
the affinity of the Fab towards tetanus toxoid 10 to
100-fold (from 6.3×10^{-8} M to 6.3×10^{-6} M). In the
35   crosses of the light chain of P3-13 with all the heavy

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chains of the HIV pannings, the productive crosses had
similar affinities to gp120 (IIIB) (2.5 X 10^7 - 6.3 X
10^{-7} M), with the exception of b14HC/P3-13LC, whose
signal was too weak for a definite determination of
the apparent binding constant. These affinities were
approximately five-fold lower than those of the
gp120-heavy chains with their original light chains.

Thus, the results show that chain shuffling is
yet another maneuver allowed _in vitro_ but not _in vivo_
which can be expected to help extend antibody
diversity beyond that of Nature. The overriding
feature of the binary system of this invention is its
ability to create large numbers (several hundred) of
directed crosses between characterized light and heavy
chains without the need for recloning individual
chains for each cross after the initial vector
construction. When used in combination with the
phage-display method and biological assays, it allows
the rapid analysis of the most interesting subset of
the pool of antigen-binding clones by chain shuffling,
with the aim of finding biologically or chemically
active antibodies. For the set of antigens studied
here, most heavy chains recombined with a number of
light chains to yield an antigen-binding Fab fragment.

These results have important implications for the
diversity of combinatorial antibody libraries. While
it is not possible to predict reliably the original _in
vivo_ combinations of light and heavy chains due to the
surprising promiscuity of individual chains,
recombinant antibody libraries take advantage of the
fact that even distantly related Fabs against the same
antigen can recombine _in vitro_ to give chain
combinations not found _in vivo_. In fact, after the
identification of a certain number of antibodies that
have been shown to possess some biological or chemical

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activity, it may be better to shuffle their individual
chains in a directed fashion than to continue sampling
randomly from the same pool of binders. By extension,
the promiscuity observed in this system indicates that
5   in libraries constructed using degenerate, chemically
synthesized oligonucleotides, there should be
considerable flexibility in which separate synthetic
heavy chains can pair with separate synthetic light
chains to generate separate antigen-binding Fab
10   fragments. The diversity of combinatorial libraries
coupled with chain-shuffling should allow wide
exploration of three dimensional space thereby solving
the problem of how to approximate molecules in the
ternary complex of antibody, substrate and cofactor.
15

7.   Deposit of Materials
        The following cell lines have been deposited on
September 30, 1992, with the American Type Culture
Collection (ATCC), 1301 Parklawn Drive, Rockville, MD,
20   USA:

            Cell Line   ATCC Accession No.
                E. coli   MT11   ATCC 69078

                E. coli   MT12   ATCC 69079
25   E. coli   MT13   ATCC 69080

        The deposits listed above, MT11, MT12 and MT13
are bacterial cells (E. coli) containing the
expression vector pComb2-3 for the respective
30   expression of the Fabs designated b11 (clone b11), b12
(clone b12), and b13 (clone b13) prepared in Example
2b. The sequences of the heavy and light chain
variable domains are listed in Figure 10 and 11,
respectively. This deposit was made with the ATCC
35   under the provisions of the Budapest Treaty on the

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International Recognition of the Deposit of
Microorganisms for the Purpose of Patent Procedure and
the Regulations thereunder (Budapest Treaty). This
assures maintenance of a viable culture for 30 years
from the date of deposit. The organisms will be made
available by ATCC under the terms of the Budapest
Treaty which assures permanent and unrestricted
availability of the progeny of the culture to the
public upon issuance of the pertinent U.S. patent or
upon laying open to the public of any U.S. or foreign
patent application, whichever comes first, and assures
availability of the progeny to one determined by the
U.S. Commissioner of Patents and Trademarks to be
entitled thereto according to 35 U.S.C. §122 and the
Commissioner's rules pursuant thereto (including 37
CFR §1.14 with particular reference to 886 OG 638).
The assignee of the present application has agreed
that if the culture deposit should die or be lost or
destroyed when cultivated under suitable conditions,
it will be promptly replaced on notification with a
viable specimen of the same culture. Availability of
the deposited strain is not to be construed as a
license to practice the invention in contravention of
the rights granted under the authority of any
government in accordance with its patent laws.

The foregoing written specification is considered
to be sufficient to enable one skilled in the art to
practice the invention. The present invention is not
to be limited in scope by the cell lines deposited,
since the deposited embodiment is intended as a single
illustration of one aspect of the invention and any
cell lines that are functionally equivalent are within
the scope of this invention. The deposit of material
does not constitute an admission that the written

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description herein contained is inadequate to enable
the practice of any aspect of the invention, including
the best mode thereof, nor is it to be construed as
limiting the scope of the claims to the specific
5   illustration that it represents. Indeed, various
modifications of the invention in addition to those
shown and described herein will become apparent to
those skilled in the art from the foregoing
description and fall within the scope of the appended
10   claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

   (i) APPLICANT:
       (A) NAME: The Scripps Research Institute
       (B) STREET: 10666 North Torrey Pines Road
       (C) CITY: La Jolla
       (D) STATE: CA
       (E) COUNTRY: USA
       (F) POSTAL CODE (ZIP): 92037
       (G) TELEPHONE: 619-554-2937
       (H) TELEFAX: 619-554-6312

   (ii) TITLE OF INVENTION: HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES
        TO HUMAN IMMUNODEFICIENCY VIRUS

   (iii) NUMBER OF SEQUENCES: 151

   (iv) COMPUTER READABLE FORM:
       (A) MEDIUM TYPE: Floppy disk
       (B) COMPUTER: IBM PC compatible
       (C) OPERATING SYSTEM: PC-DOS/MS-DOS
       (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

   (v) CURRENT APPLICATION DATA:
       (A) APPLICATION NUMBER: PCT/US93/
       (B) FILING DATE: 30-SEP-1993

   (vi) PRIOR APPLICATION DATA:
       (A) APPLICATION NUMBER: US 07/954,148
       (B) FILING DATE: 30-SEP-1992

(2) INFORMATION FOR SEQ ID NO:1:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 173 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCGCAAAT TCTATTTCAA GGAGACAGTC ATAATGAAAT ACCTATTGCC TACGGCAGCC   60

GCTGGATTGT TATTACTCGC TGCCCAACCA GCCATGGCCC AGGTGAAACT GCTCGAGATT   120

TCTAGACTAG TTACCCGTAC GACGTTCCGG ACTACGGTTC TTAATAGAAT TCG   173

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 173 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

  (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCGACGAATT CTATTAAGAA CCGTAGTCCG GAACGTCGTA CGGGTAACTA GTCTAGAAAT   60

CTCGAGCAGT TTCACCTGGG CCATGGCTGG TTGGGCAGCG AGTAATAACA ATCCAGCGGC   120

TGCCGTAGGC AATAGGTATT TCATTATGAC TGTCTCCTTG AAATAGAATT TGC   173

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 131 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

  (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGAATTCTAA ACTAGTCGCC AAGGAGACAG TCATAATGAA ATACCTATTG CCTACGGCAG   60

CCGCTGGATT GTTATTACTC GCTGCCCAAC CAGCCATGGC CGAGCTCGTC AGTTCTAGAG   120

TTAAGCGGCC G   131

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 139 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

  (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGACGGCCG CTTAACTCTA GAACTGACGA GCTCGGCCAT GGCTGGTTGG GCAGCGAGTA   60

ATAACAATCC AGCGGCTGCC GTAGGCAATA GGTATTTCAT TATGACTGTC TCCTTGGCGA   120

CTAGTTTAGA ATTCAAGCT   139

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 10 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser
1   5   10

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1               5                   10                  15

Ala Gln Pro Ala Met Ala Gln Val Lys Leu
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:7:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 23 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1               5                   10                  15

Ala Gln Pro Ala Met Ala Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:8:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 198 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: double
       (D) TOPOLOGY: circular

   (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAATTTCA   60

CACAGGAGGA AGGATCCATG AAATACCTAT TGCCTACGGC AGCCGCTGGA TTGTTATTAC   120

TCGCTGCCCA ACCAGCCATG GCCGAGCTCG GTCGGTCGGT CCTCGAGGGT CGGTCGGTCT   180

CTAGAGTTAA GCGGCCGC   198

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 198 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCGGCCGCTT AACTCTAGAG ACCGACCGAC CCTCGAGGAC CGACCGACCG AGCTCGGCCA   60

TGGCTGGTTG GGCAGCGAGT AATAACAATC CAGCGGCTGC CGTAGGCAAT AGGTATTTCA   120

TGGATCCTTC CTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAT TATACGAGCC   180

GATGATTAAT TGTCAACA   198

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: N-terminal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Lys Thr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1   5   10   15

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Ala Gln Pro Ala Met Ala Glu Leu
            20

(2) INFORMATION FOR SEQ ID NO:11:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 220 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAATTTCA   60

CACAGGAGGA AGGATCCATG AAATACCTAT TGCCTACGGC AGCCGCTGGA TTGTTATTAC   120

TCGCTGCCCA ACCAGCCATG GCCCAGGTGA AACTGCTCGA GGGTCGGTCG GTCTCTAGAC   180

GGTCGGTCGG TCACTAGTCA TCATCATCAT CATTAAGCTA   220

(2) INFORMATION FOR SEQ ID NO:12:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 220 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: double
          (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TAGCTTAATG ATGATGATGA TGACTAGTGA CCGACCGACC GTCTAGAGAC CGACCGACCC   60

TCGAGCAGTT TCACCTGGGC CATGGCTGGT TGGGCAGCGA GTAATAACAA TCCAGCGGCT   120

GCCGTAGGCA ATAGGTATTT CATGGATCCT TCCTCCTGTG TGAAATTGTT ATCCGCTCAC   180

AATTCCACAC ATTATACGAG CCGATGATTA ATTGTCAACA   220

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (v) FRAGMENT TYPE: N-terminal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Lys Thr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1   5   10   15

Ala Gln Pro Ala Met Ala Gln Val Lys Leu Leu Glu
            20   25

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (v) FRAGMENT TYPE: C-terminal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Ser His His His His His
1   5

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGCCGCAAAT TCTATTTCAA GGAGACAGTC AT   32

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 36 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AATGAAATAC CTATTGCCTA CGGCAGCCGC TGGATT   36

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTTATTACTC GCTGCCCAAC CAGCCATGGC CC   32

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 29 base pairs

                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: DNA (genomic)

             (iii) HYPOTHETICAL: NO

              (iv) ANTI-SENSE: NO

              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAGTTTCACC TGGGCCATGG CTGGTTGGG   29

(2) INFORMATION FOR SEQ ID NO:19:

               (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 40 base pairs
                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: DNA (genomic)

             (iii) HYPOTHETICAL: NO

              (iv) ANTI-SENSE: NO

              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAGCGAGTAA TAACAATCCA GCGGCTGCCG TAGGCAATAG   40

(2) INFORMATION FOR SEQ ID NO:20:

               (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 38 base pairs
                    (B) TYPE: nucleic acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: DNA (genomic)

             (iii) HYPOTHETICAL: NO

              (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTATTTCATT ATGACTGTCT CCTTGAAATA GAATTTGC   38

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 40 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGGTGAAACT GCTCGAGATT TCTAGACTAG TTACCCGTAC   40

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGAACGTCG TACGGGTAAC TAGTCTAGAA ATCTCGAG   38

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GACGTTCCGG ACTACGGTTC TTAATAGAAT TCG   33

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCGACGAATT CTATTAAGAA CCGTAGTC   28

(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGAATTCTAA ACTAGTCGCC AAGGAGACAG TCAT   34

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 36 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AATGAAATAC CTATTGCCTA CGGCAGCCGC TGGATT   36

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTTATTACTC GCTGCCCAAC CAGCCATGGC C   31

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 30 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAGCTCGTCA GTTCTAGAGT TAAGCGGCCG   30

.(2) INFORMATION FOR SEQ ID NO:29:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 48 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         .(D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTATTTCATT ATGACTGTCT CCTTGGCGAC TAGTTTAGAA TTCAAGCT   48

(2) INFORMATION FOR SEQ ID NO:30:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 40 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAGCGAGTAA TAACAATCCA GCGGCTGCCG TAGGCAATAG   40

(2) INFORMATION FOR SEQ ID NO:31:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 27 base pairs

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```
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO



      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
```

TGACGAGCTC GGCCATGGCT GGTTGGG   27

(2) INFORMATION FOR SEQ ID NO:32:

```
       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO



      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
```

TCGACGGCCG CTTAACTCTA GAAC   24

(2) INFORMATION FOR SEQ ID NO:33:

```
       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 666 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

     (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
CCATTCGTTT GTGAATATCA AGGCCAAGGC CAATCGTCTG ACCTGCCTCA ACCTCCTGTC      60

AATGCTGGCG GCGGCTCTGG TGGTGGTTCT GGTGGCGGCT CTGAGGGTGG TGGCTCTGAG     120

GGTGGCGGTT CTGAGGGTGG CGGCTCTGAG GGAGGCGGTT CCGGTGGTGG CTCTGGTTCC     180

GGTGATTTTG ATTATGAAAA GATGGCAAAC GCTAATAAGG GGGCTATGAC CGAAAATGCC     240

GATGAAAACG CGCTACAGTC TGACGCTAAA GGCAAACTTG ATTCTGTCGC TACTGATTAC     300

GGTGCTGCTA TCGATGGTTT CATTGGTGAC GTTTCCGGCC TTGCTAATGG TAATGGTGCT     360

ACTGGTGATT TTGCTGGCTC TAATTCCCAA ATGGCTCAAG TCGGTGACGG TGATAATTCA     420

CCTTTAATGA ATAATTTCCG TCAATATTTA CCTTCCCTCC CTCAATCGGT TGAATGTCGC     480

CCTTTTGTCT TTAGCGCTGG TAAACCATAT GAATTTTCTA TTGATTGTGA CAAAATAAAC     540

TTATTCGGTG TCTTTGCGTT TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG     600

TTTGCTAACA TACTGCGTAA TAAGGAGTCT TAATCATGCC AGTTCTTTTG GGTATTCCGT     660

TATTAT                                                             666
```

(2) INFORMATION FOR SEQ ID NO:34:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 211 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: unknown

   (ii) MOLECULE TYPE: protein

  (iii) HYPOTHETICAL: NO

   (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Pro Phe Val Cys Glu Tyr Gln Gly Gln Gly Gln Ser Ser Asp Leu Pro
1               5                   10                  15

Gln Pro Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
            20                  25                  30

Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
        35                  40                  45
```

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```
Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp
    50              55              60

Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala
65              70              75                      80

Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val
                85              90                      95

Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser
            100             105             110

Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn
            115             120             125

Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn
    130             135             140

Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg
145             150             155             160

Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys
                165             170             175

Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val
            180             185             190

Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn
            195             200             205

Lys Glu Ser
210
```

(2) INFORMATION FOR SEQ ID NO:35:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 48 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAGACGACTA GTGGTGGCGG TGGCTCTCCA TTCGTTTGTG AATATCAA   48

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(2) INFORMATION FOR SEQ ID NO:36:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 40 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TTACTAGCTA GCATAATAAC GGAATACCCA AAAGAACTGG   40

(2) INFORMATION FOR SEQ ID NO:37:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 36 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TATGCTAGCT AGTAACACGA CAGGTTTCCC GACTGG   36

(2) INFORMATION FOR SEQ ID NO:38:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ACCGAGCTCG AATTCGTAAT CATGGTC   27

(2) INFORMATION FOR SEQ ID NO:39:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 31 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGCTGTTGAA TTCGTGAAAT TGTTATCCGC T   31

(2) INFORMATION FOR SEQ ID NO:40:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 708 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: double
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GAGACGACTA GTGGTGGCGG TGGCTCTCCA TTCGTTTGTG AATATCAAGG CCAAGGCCAA   60

TCGTCTGACC TGCCTCAACC TCCTGTCAAT GCTGGCGGCG GCTCTGGTGG TGGTTCTGGT   120

GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT GGCGGTTCTG AGGGTGGCGG CTCTGAGGGA   180

GGCGGTTCCG GTGGTGGCTC TGGTTCCGGT GATTTTGATT ATGAAAAGAT GGCAAACGCT   240

AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC TACAGTCTGA CGCTAAAGGC   300

AAACTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCAT TGGTGACGTT   360

TCCGGCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTTG CTGGCTCTAA TTCCCAAATG   420

GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTCA ATATTTACCT   480

TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTA GCGCTGGTAA ACCATATGAA   540

TTTTCTATTG ATTGTGACAA AATAAACTTA TTCCGTGGTG TCTTTGCGTT TCTTTTATAT   600

GTTGCCACCT TTATGTATGT ATTTTCTACG TTTGCTAACA TACTGCGTAA TAAGGAGTCT   660

TAATCATGCC AGTTCTTTTG GGTATTCCGT TATTATGCTA GCTAGTAA   708

(2) INFORMATION FOR SEQ ID NO:41:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 201 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TATGCTAGCT AGTAACACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA   60

ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT   120

CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT   180

GATTACGAAT TCGAGCTCGG T   201

(2) INFORMATION FOR SEQ ID NO:42:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CAGGTGCAGC TCGAGCAGTC TGGG   24

(2) INFORMATION FOR SEQ ID NO:43:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 24 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GAGGTGCAGC TCGAGGAGTC TGGG   24

(2) INFORMATION FOR SEQ ID NO:44:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 30 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GCATGTACTA GTTTTGTCAC AAGATTTGGG   30

(2) INFORMATION FOR SEQ ID NO:45:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GACATCGAGC TCACCCAGTC TCCA   24

(2) INFORMATION FOR SEQ ID NO:46:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GAAATTGAGC TCACGCAGTC TCCA   24

(2) INFORMATION FOR SEQ ID NO:47:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 53 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCGCCGTCTA GAACTAACAC TCTCCCCTGT TGAAGCTCTT TGTGACGGGC AAG   53

(2) INFORMATION FOR SEQ ID NO:48:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 12 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: circular

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

    Ser Ile Ser Gly Pro Gly Arg Ala Phe Tyr Thr Gly
    1   5   10

(2) INFORMATION FOR SEQ ID NO:49:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GTCGTTGACC AGGCAGCCCA G   21

(2) INFORMATION FOR SEQ ID NO:50:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid

            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ATAGAAGTTG TTCAGCAGGC A   21

(2) INFORMATION FOR SEQ ID NO:51:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 17 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ATTAACCCTC ACTAAAG   17

(2) INFORMATION FOR SEQ ID NO:52:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 22 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

     (iv) ANTI-SENSE: NO

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GAATTCTAAA CTAGCTAGTT CG   22

(2) INFORMATION FOR SEQ ID NO:53:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu Glu Glu Ser Gly Thr Glu Phe Lys Pro Pro Gly Ser Ser Val Lys
1   5   10   15

Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Gly Asp Tyr Ala Ser Asn
            20   25   30

Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
            35   40   45

Ile Gly Gly Ile Thr Pro Thr Ser Gly Ser Ala Asp Tyr Ala Gln Lys
        50   55   60

Phe Gln Gly Arg Val Thr Ile Ser Ala Asp Arg Phe Thr Pro Ile Leu
65   70   75   80

Tyr Met Glu Leu Arg Ser Leu Arg Ile Glu Asp Thr Ala Ile Tyr Tyr
            85   90   95

Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
            100   105   110

Gly Ala Leu Asp Phe Trp Gly Gln Gly Thr Arg Val Phe Val Ser Pro
        115   120   125

(2) INFORMATION FOR SEQ ID NO:54:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

155

```
Leu Glu Glu Ser Gly Ala Ala Val Gln Lys Pro Gly Ser Ser Val Arg
1               5                   10              15

Val Ser Cys Gln Ala Ser Gly Gly Thr Phe Asp Asn Phe Ala Ser Asn
            20              25              30

Tyr Ala Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
        35              40              45

Met Gly Gly Ile Thr Pro Thr Ser Gly Thr Ala Thr Tyr Ser Gln Lys
    50              55              60

Phe Gln Gly Arg Val Thr Ile Ser Ala Ala Pro Leu Thr Pro Ile Ile
65              70              75              80

Tyr Met Glu Leu Arg Ser Leu Arg Asp Asp Asp Thr Ala Val Tyr Tyr
            85              90              95

Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Val
            100             105             110

Gly Ala Leu Asp Val Trp Gly Gln Gly Thr Thr Val
            115             120
```

(2) INFORMATION FOR SEQ ID NO:55:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Leu Glu Glu Ser Gly Thr Glu Phe Lys Pro Pro Gly Ser Ser Val Lys
1               5                   10              15

Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Gly Asp Tyr Ala Ser Asn
            20              25              30

Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
        35              40              45

Ile Gly Gly Ile Thr Pro Thr Ser Gly Ser Ala Asp Tyr Ala Gln Lys
    50              55              60

Phe Gln Gly Arg Val Thr Ile Ser Ala Asp Arg Phe Thr Pro Ile Leu
65              70              75              80
```

```
Tyr Met Glu Leu Arg Ser Leu Arg Ile Glu Asp Thr Ala Ile Tyr Tyr
             85                  90                  95
```

```
Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
             100                 105                 110
```

```
Gly Ala Leu Asp Phe Trp Gly Gln Gly Thr Arg Val Phe Val Ser Pro
             115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:56:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 128 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```
Leu Glu Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys
1               5                  10                  15
```

```
Val Ser Cys Lys Ala Ser Gly Gly Ile Phe Ser Asp Phe Ala Ser Asn
             20                  25                  30
```

```
Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
             35                  40                  45
```

```
Met Gly Gly Ile Thr Pro Thr Ser Gly Ser Ala Asp Tyr Ala Gln Lys
             50                  55                  60
```

```
Phe Gln Gly Arg Val Thr Ile Ser Ala Asp Ala Ala Thr Pro Arg Val
65                  70                  75                  80
```

```
Tyr Met Glu Leu Arg Ile Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe
             85                  90                  95
```

```
Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
             100                 105                 110
```

```
Gly Ala Leu Glu Val Trp Gly Gln Gly Thr Thr Val Ile Val Ser Pro
             115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:57:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 128 amino acids

```
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

    Leu Glu Glu Ser Gly Ala Ala Val Gln Lys Pro Gly Ser Ser Val Arg
    1               5                   10                  15

    Val Ser Cys Gln Ala Ser Gly Gly Thr Phe Asp Asn Phe Ala Ser Asn
                20                  25                  30

    Tyr Ala Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
                35              40                  45

    Met Gly Gly Ile Thr Pro Thr Ser Gly Thr Ala Thr Tyr Ser Gln Lys
        50                  55                  60

    Phe Gln Gly Arg Val Thr Ile Ser Ala Ala Pro Leu Thr Pro Ile Ile
    65                  70                  75                  80

    Tyr Met Glu Leu Arg Ser Leu Arg Asp Asp Asp Thr Ala Val Tyr Tyr
                85                  90                  95

    Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Val
                100                 105                 110

    Gly Ala Leu Asp Val Trp Gly Gln Gly Thr Thr Val Ile Val Ser Ser
            115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:58:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
    Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys
    1               5                   10                  15

    Val Ser Cys Lys Thr Ser Gly Gly Thr Phe Ser Asp Tyr Ala Ser Asn
                20                  25                  30
```

158

```
His Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
        35              40              45

Met Gly Gly Ile Thr Pro Thr Ser Gly Thr Ala Asp Tyr Ala Gln Lys
        50              55              60

Phe Gln Ala Arg Val Thr Ile Ser Ala His Glu Phe Thr Pro Ile Val
65              70              75              80

Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Gln His Ala Thr Tyr Tyr
            85              90              95

Cys Ala Thr Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
            100             105             110

Gly Ala Leu Asp Ile Trp Gly Gln Gly Thr Thr Val Ile Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:59:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 128 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
Leu Glu Glu Ser Gly Gly Arg Leu Val Lys Pro Gly Gly Ser Leu Arg
1               5               10              15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
            20              25              30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35              40              45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
    50              55              60

Gly Arg Phe Ser Ile Ser Arg Asn Asp Leu Glu Asp Lys Met Phe Leu
65              70              75              80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85              90              95

Thr Lys Tyr Pro Arg Tyr Ser Asp Met Val Thr Gly Val Arg Asn His
            100             105             110
```

```
Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:60:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
Leu Glu Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1               5               10              15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
        20              25              30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35              40              45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
        50              55              60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Leu Phe Leu
65              70              75              80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
                85              90              95

Thr Lys Tyr Pro Arg Tyr Phe Asp Met Met Ala Gly Val Arg Asn His
            100             105             110

Phe Tyr Met Asp Val Trp Gly Thr Gly Thr Thr Val Ile Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:61:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

160

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1   5   10   15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
                20   25   30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35   40   45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
    50   55   60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Leu Phe Leu
65   70   75   80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85   90   95

Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Ala Gly Val Arg Asn His
            100   105   110

Leu Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
        115   120   125

(2) INFORMATION FOR SEQ ID NO:62:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 128 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Leu Glu Glu Ser Gly Gly Arg Leu Val Lys Pro Gly Gly Ser Leu Arg
1   5   10   15

Leu Ser Cys Glu Ala Ser Gly Phe Thr Phe Thr Asn Ser Trp Met Thr
                20   25   30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35   40   45

Lys Arg Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
    50   55   60

```
Gly Arg Phe Ser Ile Ser Arg Asn Asp Leu Glu Asp Lys Met Phe Leu
65              70              75              80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85              90              95

Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Thr Gly Val Arg Asn His
            100             105             110

Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:63:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```
Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1               5               10              15

Leu Ser Cys Glu Ser Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
            20              25              30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
            35              40              45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
        50              55              60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Leu Phe Leu
65              70              75              80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85              90              95

Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Ala Gly Val Arg Asn His
            100             105             110

Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:64:

162

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

    Leu Glu Glu Ser Gly Gly Arg Leu Val Lys Pro Gly Gly Ser Leu Arg
    1   5   10   15

    Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
                20   25   30

    Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
            35   40   45

    Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
        50   55   60

    Gly Arg Phe Ser Ile Ser Arg Asn Asp Leu Glu Asp Lys Met Phe Leu
    65   70   75   80

    Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
                85   90   95

    Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Thr Gly Val Arg Asn His
                100   105   110

    Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
            115   120   125

(2) INFORMATION FOR SEQ ID NO:65:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 128 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

    Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
    1   5   10   15

```
Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
            20              25              30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35              40              45

Lys Ser Lys Phe Asp Gly Gly Ser Ser His Tyr Pro Gly Pro Val Glu
    50              55              60

Gly Arg Phe Thr Ile Ser Arg Asn Tyr Ile Glu Asp Lys Leu Phe Leu
65              70              75              80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85              90              95

Thr Lys Tyr Pro Arg Tyr Tyr Asp Met Met Arg Gly Val Arg Asn His
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:66:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

```
Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
1               5               10              15

Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn Phe Val Ile His
            20              25              30

Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp Met Gly Trp Ile
        35              40              45

Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys Phe Gln Asp Arg
    50              55              60

Val Thr Phe Thr Ala Asp Thr Ser Ala Asn Thr Ala Tyr Met Glu Leu
65              70              75              80

Arg Ser Leu Arg Ser Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Val
            85              90              95
```

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```
Gly Pro Tyr Ser Trp Asp Asp Ser Pro Gln Asp Asn Tyr Tyr Met Asp
        100                 105                 110

Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:67:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

```
Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
1               5               10                  15

Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn Phe Val Ile His
        20                  25                  30

Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp Met Gly Trp Ile
        35                  40                  45

Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys Phe Gln Asp Arg
        50                  55                  60

Val Thr Phe Thr Ala Asp Thr Asp Ala Asn Thr Ala Tyr Met Glu Leu
65                  70                  75                  80

Arg Ser Leu Arg Ser Ala Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Val
                85                  90                  95

Gly Pro Tyr Thr Trp Asp Asp Ser Pro Gln Asp Asn Tyr Tyr Met Asp
        100                 105                 110

Val Trp Gly Lys Gly Thr Lys Val Ile Val Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:68:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```
Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
1               5                   10                  15

Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn Phe Val Ile His
            20                  25              30

Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp Met Gly Trp Ile
        35                  40              45

Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys Phe Gln Asp Arg
    50                  55              60

Val Thr Phe Thr Ala Asp Thr Asp Ala Asn Thr Ala Tyr Met Glu Leu
65                  70              75                  80

Arg Ser Leu Arg Ser Thr Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Val
            85                  90                  95

Gly Pro Tyr Thr Trp Asp Asp Ser Pro Gln Asp Asn Tyr Tyr Met Asp
            100             105             110

Val Trp Gly Lys Gly Thr Lys Val Ile Val Ser Ser
        115             120
```

(2) INFORMATION FOR SEQ ID NO:69:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 130 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```
Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1               5                   10                  15

Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Ser Ser Ala Trp Met Ala
            20                  25              30

Trp Val Arg Gln Ala Pro Gly Arg Gly Leu Glu Trp Val Gly Leu Ile
        35                  40              45

Lys Ser Lys Ala Asp Gly Glu Thr Thr Asp Tyr Ala Thr Pro Val Lys
    50                  55              60
```

```
Gly Arg Phe Ser Ile Ser Arg Asn Asn Leu Glu Asp Thr Val Tyr Leu
65              70              75              80

Gln Met Asp Ser Leu Arg Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
            85              90              95

Thr Gln Lys Pro Arg Tyr Phe Asp Leu Leu Ser Gly Gln Tyr Arg Arg
        100             105             110

Val Ala Gly Ala Phe Asp Val Trp Gly His Gly Thr Thr Val Thr Val
        115             120             125

Ser Pro
130
```

(2) INFORMATION FOR SEQ ID NO:70:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 130 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

```
Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Ala Gly Gly Ser Leu Arg
1               5               10              15

Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Ser Ser Ala Trp Met Ala
            20              25              30

Trp Val Gly Gln Ala Pro Gly Arg Gly Leu Glu Trp Val Gly Leu Ile
            35              40              45

Lys Ser Lys Ala Asp Gly Glu Thr Thr Asp Tyr Ala Thr Pro Val Lys
        50              55              60

Gly Arg Phe Ser Ile Ser Arg Asn Asn Leu Glu Asp Thr Val Tyr Leu
65              70              75              80

Gln Met Asp Ser Leu Arg Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
            85              90              95

Thr Gln Lys Pro Arg Tyr Phe Asp Leu Leu Ser Gly Gln Tyr Arg Arg
        100             105             110

Val Ala Gly Ala Phe Asp Val Trp Gly His Gly Thr Thr Val Thr Val
        115             120             125
```

Ser Pro
    130

(2) INFORMATION FOR SEQ ID NO:71:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 130 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

    Leu Glu Glu Ser Gly Gly Gly Leu Ile Lys Pro Gly Gly Ser Leu Arg
    1   5   10   15

    Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Ser Ser Ala Trp Met Thr
                20   25   30

    Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile
            35   40   45

    Lys Ser Lys Ala Asp Gly Glu Thr Thr Asp Tyr Ala Thr Pro Val Lys
        50   55   60

    Gly Arg Phe Thr Ile Ser Arg Asn Asn Leu Glu Asn Thr Val Tyr Leu
    65   70   75   80

    Gln Met Asp Ser Leu Arg Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
                85   90   95

    Thr Gln Lys Pro Ser Tyr Tyr Asn Leu Leu Ser Gly Gln Tyr Arg Arg
                100   105   110

    Val Ala Gly Ala Phe Asp Val Trp Gly His Gly Thr Thr Val Thr Val
            115   120   125

    Ser Pro

(2) INFORMATION FOR SEQ ID NO:72:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Leu Glu Glu Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
1   5   10   15

Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Asn Tyr Ala Met His
            20   25   30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
        35   40   45

Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
    50   55   60

Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
65   70   75   80

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
            85   90   95

Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
            100   105   110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115   120   125

(2) INFORMATION FOR SEQ ID NO:73:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Thr Ser Leu Arg
1   5   10   15

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Ala Met His
            20   25   30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
        35   40   45

Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
    50   55   60

```
Phe Ser Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Glu Met
65                  70              75                  80

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
            85              90              95

Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
            100             105             110

Asp Tyr Trp Gly Gln Gly Ala Leu Val Thr Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:74:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

```
Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
1               5               10              15

Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Asn Tyr Ala Met His
            20              25              30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
        35              40              45

Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
    50              55              60

Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
65                  70              75                  80

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
            85              90              95

Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
            100             105             110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:75:

    (i) SEQUENCE CHARACTERISTICS:

170

```
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

    Leu Glu Glu Ser Gly Glu Ala Val Val Gln Pro Gly Thr Ser Leu Arg
    1               5               10              15

    Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Ala Met His
            20              25              30

    Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
            35              40              45

    Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
        50              55              60

    Phe Ser Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Glu Met
    65              70              75              80

    Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
                85              90              95

    Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
                100             105             110

    Asp Tyr Trp Gly Gln Gly Ala Leu Val Thr Val Ser Ser
            115             120             125
```

(2) INFORMATION FOR SEQ ID NO:76:

```
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

    Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
    1               5               10              15

    Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Ala Met His
            20              25              30
```

```
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
     35                  40                45
```

```
Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
     50                  55                60
```

```
Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
65                  70                75                80
```

```
Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
              85                90                95
```

```
Ile Gly Leu Lys Ala Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
              100               105               110
```

```
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
         115                120               125
```

(2) INFORMATION FOR SEQ ID NO:77:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```
Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
1             5                 10                15
```

```
Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Asn Tyr Ala Met His
         20                25                30
```

```
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
     35                  40                45
```

```
Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
     50                  55                60
```

```
Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
65                  70                75                80
```

```
Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
              85                90                95
```

```
Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
              100               105               110
```

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```
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:78:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 128 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

```
Leu Glu Gln Ser Gly Gly Gly Val Val Lys Pro Gly Gly Ser Leu Arg
1               5               10              15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Pro Asn Ala Trp Met Thr
            20              25              30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35              40              45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
    50              55              60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Val Phe Leu
65              70              75              80

Gln Met Asn Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85              90              95

Thr Arg Tyr Pro Arg Tyr Ser Glu Met Met Gly Gly Val Arg Lys His
            100             105             110

Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ser Val Ser Ser
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:79:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 128 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

```
Leu Glu Glu Ser Gly Gly Gly Val Val Lys Pro Gly Gly Ser Leu Arg
1               5                   10                  15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Pro Asn Ala Trp Met Thr
            20              25              30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
        35              40              45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
    50              55              60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Val Phe Leu
65              70              75              80

Gln Met Asn Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
            85              90              95

Thr Arg Tyr Pro Arg Tyr Ser Glu Met Met Gly Gly Val Arg Lys His
            100             105             110

Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ser Val Ser Ser
            115             120             125
```

(2) INFORMATION FOR SEQ ID NO:80:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 122 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

```
Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg
1               5                   10                  15

Val Ser Cys Glu Ala Ser Gly Phe Thr Phe Ser Ser Tyr Glu Met Asn
            20              25              30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gln Ile
        35              40              45

Ser Ser Ser Gly Ser Arg Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg
    50              55              60
```

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Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Glu Met
65   70   75   80

Thr Ser Leu Arg Val Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly
                85   90   95

Arg Arg Leu Val Thr Phe Gly Gly Val Val Ser Gly Gly Asn Ile Trp
            100   105   110

Gly Gln Gly Thr Met Val Thr Val Ser Ser
        115   120

(2) INFORMATION FOR SEQ ID NO:81:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 126 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg
1   5   10   15

Leu Ser Cys Ala Gly Ser Gly Phe Asn Phe Ser Asp Asp Thr Met His
            20   25   30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val Ile
        35   40   45

Ser Tyr Glu Gly Ser Asp Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
    50   55   60

Phe Thr Ile Ser Arg Asp Asn Ser Glu Asn Thr Leu Tyr Leu Gln Met
65   70   75   80

Asp Ser Leu Arg Ala Asp Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Asn
            85   90   95

Thr Arg Glu Asn Ile Glu Ala Asp Gly Thr Ala Tyr Tyr Ser Tyr Tyr
            100   105   110

Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser
        115   120   125

(2) INFORMATION FOR SEQ ID NO:82:

    (i) SEQUENCE CHARACTERISTICS:

```
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

    Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
    1               5                   10              15

    Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Tyr Leu Ala
                20                  25                  30

    Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Arg Leu Leu Ile Tyr Ala
            35                  40                  45

    Ala Ser Thr Leu Gln Pro Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
        50                  55                  60

    Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
    65                  70                  75                  80

    Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Arg Thr Phe
                    85                  90                  95

    Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
                100                 105
```

(2) INFORMATION FOR SEQ ID NO:83:

```
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 106 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

    Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Ile Gly Asp Arg
    1               5                   10              15

    Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asn Asn Tyr Leu Ala
                20                  25                  30

    Trp Tyr Gln Gln Arg Pro Gly Lys Val Pro Arg Leu Leu Ile Tyr Ala
            35                  40                  45
```

```
Ala Ser Thr Leu Gln Ser Gly Val Pro Thr Arg Phe Ser Gly Ser Gly
    50                  55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65                  70                  75                  80

Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Val Pro Arg Thr Phe
                85                  90                  95

Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:84:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5                   10                  15

Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Tyr Leu Ala
            20                  25                  30

Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Ala
        35                  40                  45

Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
    50                  55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65                  70                  75                  80

Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Arg Thr Phe
                85                  90                  95

Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:85:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 106 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Ile Gly Asp Arg
1               5                   10              15

Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asn Asn Tyr Leu Ala
            20              25              30

Trp Tyr Gln Gln Arg Pro Gly Lys Ala Pro Asn Leu Leu Ile Tyr Ala
        35              40              45

Ala Ser Thr Leu Gln Ser Gly Val Pro Pro Arg Phe Ser Gly Ser Gly
    50              55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65              70              75              80

Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Val Pro His Thr Phe
                85              90              95

Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
            100             105
```

(2) INFORMATION FOR SEQ ID NO:86:

 (i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 108 amino acids
  (B) TYPE: amino acid
  (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1               5                   10              15

Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ile Ser Asn Tyr Leu
            20              25              30

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
        35              40              45

Gly Val Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser
    50              55              60
```

```
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
65              70              75              80

Asp Phe Ala Val Tyr Ser Cys Gln Gln Tyr Gly Thr Ser Pro Trp Thr
            85              90              95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
        100             105
```

(2) INFORMATION FOR SEQ ID NO:87:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1               5               10              15

Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Asn Asn Tyr Leu
            20              25              30

Ala Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
        35              40              45

Gly Ala Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Gly Thr Ala Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65              70              75              80

Asp Val Ala Ile Tyr Tyr Cys Gln Gln Tyr His Ser Ser Pro Tyr Thr
            85              90              95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
        100             105
```

(2) INFORMATION FOR SEQ ID NO:88:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1               5               10              15

Ala Thr Leu Ser Cys Arg Ala Ser His Arg Val Asn Asn Asn Phe Leu
            20              25              30

Ala Trp Tyr Gln Gln Lys Pro Gln Ala Pro Arg Leu Leu Ile Ser Gly
        35              40              45

Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly
    50              55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Asp Asp
65              70              75              80

Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Asp Ser Pro Leu Tyr Ser
            85              90              95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
            100             105
```

(2) INFORMATION FOR SEQ ID NO:89:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 105 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

```
Glu Leu Thr Gln Ser Pro Ala Ser Val Ser Ala Ser Val Gly Asp Thr
1               5               10              15

Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile His Asn Trp Leu Ala
            20              25              30

Trp Tyr Gln Gln Gln Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
        35              40              45

Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Arg Gly
    50              55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65              70              75              80
```

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    Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Ser Phe Pro Lys Phe Gly
                85   90   95

    Pro Gly Thr Val Val Asp Ile Lys Arg
            100   105

(2) INFORMATION FOR SEQ ID NO:90:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

    Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
    1   5   10   15

    Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Leu Ser Asn Asn Tyr Leu
            20   25   30

    Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
          35   40   45

    Gly Ser Ser Thr Arg Gly Thr Gly Ile Pro Asp Arg Phe Ser Gly Gly
       50   55   60

    Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
    65   70   75   80

    Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Asn Ser Val Tyr Thr
            85   90   95

    Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
            100   105

(2) INFORMATION FOR SEQ ID NO:91:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 104 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

```
Gln Ser Pro Asp Thr Leu Ser Leu Asn Pro Gly Glu Arg Ala Thr Leu
1               5               10              15

Ser Cys Arg Ala Ser His Arg Ile Ser Ser Lys Arg Leu Ala Trp Tyr
            20              25              30

Gln His Lys Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr Val Cys Pro
        35              40              45

Asn Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
    50              55              60

Thr Asp Phe Thr Leu Thr Tyr Ser Arg Leu Glu Pro Glu Asp Phe Ala
65              70              75              80

Met Tyr Tyr Cys Gln Tyr Tyr Gly Gly Ser Ser Tyr Thr Phe Gly Gln
            85              90              95

Gly Thr Lys Val Glu Ile Thr Arg
            100
```

(2) INFORMATION FOR SEQ ID NO:92:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 104 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

```
Gln Ser Pro Ser His Leu Ser Leu Ser Pro Gly Glu Arg Ala Ile Leu
1               5               10              15

Ser Cys Arg Ala Ser Gln Arg Val Ser Ala Pro Tyr Leu Ala Trp Tyr
            20              25              30

Gln Gln Arg Pro Gly Gln Ala Pro Arg Leu Val Ile Tyr Gly Ala Ser
        35              40              45

Thr Arg Ala Thr Asp Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
    50              55              60

Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala
65              70              75              80

Ile Tyr Tyr Cys Gln Val Tyr Gly Gln Ser Pro Val Leu Phe Gly Gln
            85              90              95
```

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```
Gly Thr Lys Leu Glu Met Lys Arg
            100
```

(2) INFORMATION FOR SEQ ID NO:93:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 105 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```
Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Asp Arg Ala Thr Leu
1               5                   10                  15

Ser Cys Arg Ala Ser Gln Ser Leu Ser Ser Ser Phe Leu Ala Trp Tyr
            20                  25                  30

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ser Ala Ser
        35                  40                  45

Met Arg Ala Thr Gly Ile Pro Asp Arg Phe Arg Gly Ser Val Ser Gly
        50                  55                  60

Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu Asp Phe Ala
65                  70                  75                  80

Val Tyr Tyr Cys Gln Arg Phe Gly Thr Ser Pro Leu Tyr Thr Phe Gly
                85                  90                  95

Gln Gly Thr Lys Leu Glu Met Lys Arg
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:94:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 104 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

```
Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu
1               5                   10                  15
```

183

```
Ser Cys Arg Ala Ser Gln Ser Phe Ser Ser Asn Phe Leu Ala Trp Tyr
        20              25              30

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Val His Pro
        35              40              45

Asn Arg Ala Thr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
    50              55              60

Thr Asp Phe Thr Leu Thr Ile Arg Arg Leu Glu Pro Glu Asp Phe Ala
65              70              75              80

Val Tyr Tyr Cys Gln Gln Tyr Gly Ala Ser Leu Val Ser Phe Gly Pro
            85              90              95

Gly Thr Lys Val His Ile Lys Arg
            100
```

(2) INFORMATION FOR SEQ ID NO:95:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1               5               10              15

Ala Thr Phe Ser Cys Arg Ser Ser His Ser Ile Arg Ser Arg Arg Val
        20              25              30

Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
        35              40              45

Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Val Glu Pro Glu
65              70              75              80

Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
            85              90              95

Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys Arg Thr
            100             105
```

(2) INFORMATION FOR SEQ ID NO:96:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Thr Pro Gly Glu Arg
1               5                   10                  15

Ala Thr Leu Ser Cys Arg Thr Ser His Ser Ile Arg Ser Arg Arg Leu
                20                  25                  30

Ala Trp Tyr Gln Val Lys Gly Gly Gln Ala Pro Arg Leu Leu Ile Tyr
            35                  40                  45

Gly Val Ser Asn Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
        50                  55                  60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
65                  70                  75                  80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Arg Tyr Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
                100                 105
```

(2) INFORMATION FOR SEQ ID NO:97:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

```
Glu Leu Thr Gln Ala Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1               5                   10                  15

Ala Thr Phe Ser Cys Arg Ser Ser His Ser Ile Arg Ser Arg Arg Val
                20                  25                  30
```

```
Arg Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
        35              40              45

Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Val Glu Pro Glu
65              70              75              80

Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
                85              90              95

Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys Arg
            100             105
```

(2) INFORMATION FOR SEQ ID NO:98:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

```
Glu Leu Thr Gln Ala Pro Gly Thr Leu Ser Leu Ser Pro Gly Asp Arg
1               5               10              15

Ala Thr Phe Ser Cys Arg Ser Ser His Asn Ile Arg Ser Arg Arg Val
            20              25              30

Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
        35              40              45

Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu
65              70              75              80

Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
                85              90              95

Phe Gly Gln Gly Thr Lys Leu Asp Phe Lys Arg Thr
            100             105
```

(2) INFORMATION FOR SEQ ID NO:99:

    (i) SEQUENCE CHARACTERISTICS:

```
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

    Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
    1               5                   10                  15

    Ala Thr Leu Ser Cys Arg Ala Gly Gln Ser Ile Ser Ser Asn Tyr Leu
                20                  25                  30

    Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
            35                  40                  45

    Gly Ala Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser
        50                  55                  60

    Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Ser Arg Leu Glu Pro Glu
    65                  70                  75                  80

    Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Thr Ser Pro Tyr Thr
                85                  90                  95

    Phe Gly Gln Gly Thr Gln Leu Asp Ile Lys Arg Thr
                100                 105
```

(2) INFORMATION FOR SEQ ID NO:100:

```
    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 104 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

    Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu
    1               5                   10                  15

    Ser Cys Arg Ala Ser Gln Ser Leu Ser Asn Asn Tyr Leu Ala Trp Tyr
                20                  25                  30

    Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ser Ser
            35                  40                  45
```

```
Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Gly Gly Ser Gly
    50              55              60

Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala
65              70              75              80

Val Tyr Tyr Cys Gln Gln Tyr Gly Asn Ser Val Tyr Thr Phe Gly Gln
            85              90              95

Gly Thr Lys Leu Glu Ile Lys Arg
            100
```

(2) INFORMATION FOR SEQ ID NO:101:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 106 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5               10              15

Val Thr Ile Thr Cys Arg Thr Ser Gln Gly Ile Ser Asn Tyr Leu Ala
            20              25              30

Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Gly
        35              40              45

Ala Ser Thr Leu Gln Ser Gly Gly Pro Ser Arg Phe Ser Gly Ser Gly
    50              55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro Glu Asp
65              70              75              80

Val Ala Thr Tyr Ser Cys Gln Asn Tyr Asp Ser Ala Pro Trp Thr Phe
            85              90              95

Gly Gln Gly Thr Lys Val Asp Ile Lys Arg
            100             105
```

(2) INFORMATION FOR SEQ ID NO:102:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5                   10                  15

Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Asn
            20                  25                  30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
        35                  40                  45

Ala Ser Ser Leu Gln Arg Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
    50                  55                  60

Ser Gly Thr Asp Phe Thr Leu Ser Ile Ser Ser Leu Gln Pro Glu Asp
65                  70                  75                  80

Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Ile Pro Pro Leu Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
                100                 105
```

(2) INFORMATION FOR SEQ ID NO:103:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 107 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5                   10                  15

Val Thr Ile Thr Cys Arg Ala Ser Gln Asn Ile Asn Asn Tyr Leu Asn
            20                  25                  30

Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile His Thr
        35                  40                  45

Ala Phe Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Thr Ala
    50                  55                  60
```

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```
Ser Gly Thr Glu Phe Thr Leu Thr Ile Arg Ser Leu Gln Pro Glu Asp
65                  70                  75                  80

Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr Thr Phe
                85                  90                  95

Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
                100                 105
```

(2) INFORMATION FOR SEQ ID NO:104:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5                   10                  15

Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
                20                  25                  30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
        35                  40                  45

Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
        50                  55                  60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65                  70                  75                  80

Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr Thr Phe
                85                  90                  95

Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
                100                 105
```

(2) INFORMATION FOR SEQ ID NO:105:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1   5   10   15

Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
            20   25   30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
        35   40   45

Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
    50   55   60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65   70   75   80

Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Gln Thr Phe
            85   90   95

Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
            100   105

(2) INFORMATION FOR SEQ ID NO:106:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 104 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
1   5   10   15

Thr Cys Arg Ala Ser Gln Thr Ile Ser Ser Tyr Leu Asn Trp Tyr Gln
            20   25   30

Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser
        35   40   45

Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Gly Gly Ser Gly Thr
    50   55   60

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
65   70   75   80

```
Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly
              85                  90                  95

Thr Lys Leu Glu Ile Lys Arg Thr
              100
```

(2) INFORMATION FOR SEQ ID NO:107:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5               10                  15

Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn
              20                  25                  30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp
          35                  40                  45

Ala Ser Asn Ser Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
      50                  55                  60

Ser Gly Arg Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp
65                  70                  75                  80

Val Ala Thr Tyr Tyr Cys Gln Gln His Gln Asn Val Pro Leu Thr Phe
              85                  90                  95

Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
              100                 105
```

(2) INFORMATION FOR SEQ ID NO:108:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5                   10              15

Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn His Leu Asn
            20              25              30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp
        35              40              45

Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
    50              55              60

Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp
65              70              75              80

Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Asn Leu Pro Leu Thr Phe
            85              90              95

Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
            100             105
```

(2) INFORMATION FOR SEQ ID NO:109:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

```
Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1               5                   10              15

Ile Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Asn Asn Tyr Leu Asn
            20              25              30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Gly
        35              40              45

Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
    50              55              60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
65              70              75              80

Phe Ala Thr Tyr Phe Cys Gln Gln Ser Tyr Asn Thr Pro Pro Trp Thr
            85              90              95
```

```
        Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
                    100                 105
```

(2) INFORMATION FOR SEQ ID NO:110:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 108 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1               5               10                  15

Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Asn Ser Asn Tyr Leu
            20                  25                  30

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Thr Pro Arg Val Val Ile Tyr
        35                  40                  45

Ser Thr Ser Arg Arg Ala Thr Gly Val Pro Asp Arg Phe Ser Gly Ser
        50                  55                  60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
65                  70                  75                  80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Gly Asp Ala Gln Tyr Thr
                85                  90                  95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:111:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 93 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

```
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Asn Ser Asn
1               5               10                  15
```

```
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Thr Pro Arg Val Val
            20              25              30

Ile Tyr Ser Thr Ser Arg Arg Ala Thr Gly Val Pro Asp Arg Phe Ser
        35              40              45

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
    50              55              60

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Gly Asp Ala Gln
65              70              75              80

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
                85              90
```

(2) INFORMATION FOR SEQ ID NO:112:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 104 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

```
Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly Asp Thr Val Thr
1               5               10              15

Phe Thr Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr
            20              25              30

His Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Ser Asp Ala Ser
        35              40              45

Asp Leu Glu Ile Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Ala
    50              55              60

Thr Tyr Phe Ser Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Gly
65              70              75              80

Thr Tyr Tyr Cys Gln Gln Tyr Ala Asp Leu Ile Thr Phe Gly Gly Gly
                85              90              95

Thr Lys Val Glu Ile Lys Arg Thr
            100
```

(2) INFORMATION FOR SEQ ID NO:113:

     (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 96 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val
1   5   10   15

Gly Thr Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
            20   25   30

Leu Leu Ile Phe Asp Ala Ser Thr Arg Asp Thr Tyr Ile Pro Asp Thr
            35   40   45

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ala Leu Thr Ile Ser Ser
        50   55   60

Leu Gln Ser Glu Asp Phe Gly Phe Tyr Tyr Cys Gln Gln Tyr Asp Asn
65   70   75   80

Trp Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Val Lys Arg Thr
                85   90   95

(2) INFORMATION FOR SEQ ID NO:114:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Asp Arg
1   5   10   15

Ala Thr Phe Ser Cys Arg Ser Ser His Asn Ile Arg Ser Arg Arg Val
            20   25   30

Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
        35   40   45

Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser

```
    50                    55                    60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu
65                    70                    75                    80

Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
                  85                    90                    95

Phe Gly Gln Gly Thr Lys Leu Asp Phe Lys Arg
                  100                   105
```

(2) INFORMATION FOR SEQ ID NO:115:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1                 5                   10                    15

Ala Thr Phe Ser Cys Arg Ser Ser His Asn Ile Arg Ser Arg Arg Val
                  20                  25                    30

Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
              35                    40                    45

Gly Val Ser Asn Arg Ala Thr Gly Ile Ser Asp Arg Phe Ser Gly Ser
          50                    55                    60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu
65                    70                    75                    80

Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
                  85                    90                    95

Phe Gly Gln Gly Thr Lys Leu Asp Phe Lys Arg
                  100                   105
```

(2) INFORMATION FOR SEQ ID NO:116:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

Glu Leu Thr Gln Ser Pro Asp Thr Leu Ser Leu Asn Val Gly Glu Arg
1   5   10   15

Ala Thr Leu Ser Cys Arg Ala Ser His Arg Ile Ser Ser Arg Arg Leu
            20   25   30

Ala Trp Tyr Gln His Lys Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr
        35   40   45

Gly Val Ser Ser Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
    50   55   60

Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Ser Arg Leu Glu Pro Glu
65   70   75   80

Asp Phe Ala Met Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
            85   90   95

Phe Gly Gln Gly Thr Lys Val Asp Ile Lys Arg
            100   105

(2) INFORMATION FOR SEQ ID NO:117:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

Glu Leu Thr Gln Ser Pro Asp Thr Leu Ser Leu Asn Ala Gly Glu Arg
1   5   10   15

Ala Thr Leu Ser Cys Arg Ala Ser His Arg Ile Ser Ser Arg Arg Leu
            20   25   30

Ala Trp Tyr Gln His Lys Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr
        35   40   45

Gly Val Ser Asn Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
    50   55   60

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```
Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Ser Arg Leu Glu Pro Glu
65                  70              75                  80


Asp Phe Ala Ile Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
                85              90                  95


Phe Gly Gln Gly Thr Thr Val Asp Ile Lys Arg
                100                105
```

(2) INFORMATION FOR SEQ ID NO:118:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

```
Glu Leu Thr Gln Ser Pro Asp Thr Leu Ser Leu Asn Thr Gly Glu Arg
1               5               10                  15


Ala Thr Leu Ser Cys Arg Ala Ser His Arg Ile Gly Ser Arg Arg Leu
                20              25                  30


Ala Trp Tyr Gln His Arg Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr
            35              40                  45


Gly Val Ser Asn Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
        50              55                  60


Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
65                  70              75                  80


Asp Phe Ala Ile Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
                85              90                  95


Phe Gly Gln Gly Thr Lys Val Asp Ile Lys Arg
                100                105
```

(2) INFORMATION FOR SEQ ID NO:119:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Thr Pro Gly Glu Arg
1   5   10   15

Ala Ile Leu Ser Cys Lys Thr Ser His Asn Ile Trp Ser Arg Arg Leu
              20   25   30

Ala Trp Tyr Gln Leu Lys Ser Gly Gln Ala Pro Arg Leu Leu Ile Tyr
         35   40·   45

Gly Val Ser Lys Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
         50   55   60

Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Arg Val Glu Pro Glu
65   70   75   80

Asp Phe Ala Val Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ala Tyr Thr
                   85   90   95

Phe Gly Gln Gly Thr Lys Leu Asp Ile Lys Arg
              100   105

(2) INFORMATION FOR SEQ ID NO:120:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Thr Pro Gly Glu Arg
1   5   10   15

Ala Ile Leu Ser Cys Lys Thr Ser His Asn Ile Trp Ser Arg Arg Leu
              20   25   30

Ala Trp Tyr Gln Leu Lys Ser Gly Gln Ala Pro Arg Leu Leu Ile Tyr
         35   40   45

Gly Val Ser Lys Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
         50   55   60

Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Arg Val Glu Pro Glu
65   70   75   80

200

```
Asp Phe Ala Val Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ala Tyr Thr
                85                  90              95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
            100             105
```

(2) INFORMATION FOR SEQ ID NO:121:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Ser Thr Pro Gly Glu Arg
1               5               10              15

Ala Ile Leu Ser Cys Lys Thr Ser His Asn Ile Trp Ser Arg Arg Leu
                20              25              30

Ala Trp Tyr Gln Val Lys Ser Gly Leu Pro Pro Arg Leu Leu Ile His
        35              40              45

Gly Val Ser Arg Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Ala Arg Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Ala
65              70              75              80

Asp Phe Ala Val Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Ser
                85              90              95

Phe Gly Gln Gly Thr Lys Leu Asp Phe Asn Arg
            100             105
```

(2) INFORMATION FOR SEQ ID NO:122:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

```
Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Asn Pro Gly Glu Arg
1               5               10              15

Ala Val Leu Ser Cys Arg Thr Ser Arg Asn Ile Trp Ser Arg Arg Leu
            20              25              30

Ala Trp Tyr Gln Val Arg Arg Gly Gln Ala Pro Arg Leu Leu Ile His
        35              40              45

Gly Val Ser Lys Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
    50              55              60

Gly Ser Ala Arg Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
65              70              75              80

Asp Phe Ala Val Tyr Phe Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
            85              90              95

Phe Gly Gln Gly Asn Lys Leu Asp Ile Arg Arg
            100             105
```

(2) INFORMATION FOR SEQ ID NO:123:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 126 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Pro Pro Lys
    50              55              60

Phe Gln Asp Arg Val Ser Leu Thr Arg Asp Thr Ser Ala Gly Thr Val
65              70              75              80

Tyr Leu Glu Leu Thr Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
            85              90              95
```

```
Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100                 105                 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:124:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
        50              55              60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65              70              75              80

Tyr Leu Glu Leu Arg Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
            85              90              95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Arg Gly Thr Thr Val Thr
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:125:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
    50              55              60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65              70              75              80

Tyr Leu Glu Leu Arg Ser Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
            85              90              95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
        115             120
```

(2) INFORMATION FOR SEQ ID NO:126:

       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 124 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Ile Ser Cys Gln Ala Ser Gly Tyr Arg Phe Thr Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly Gln Arg Pro Glu Trp
        35              40              45

Met Gly Trp Phe Asn Pro Ala Asn Gly Ile Lys Glu Ile Ser Pro Lys
    50              55              60
```

```
Phe Gln Asp Arg Val Ser Phe Thr Gly Asp Thr Ser Ala Ser Thr Ala
65              70              75              80

Tyr Val Glu Leu Arg Asn Leu Arg Ser Ala Asp Thr Ala Val Tyr Tyr
                85              90              95

Cys Ala Arg Val Gly Pro Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
        115             120
```

(2) INFORMATION FOR SEQ ID NO:127:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
    50              55              60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65              70              75              80

Tyr Leu Glu Leu Arg Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
                85              90              95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Phe Pro Gln Asp Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
        115             120
```

(2) INFORMATION FOR SEQ ID NO:128:

    (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 125 amino acids
    (B) TYPE: amino acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Leu Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
        50              55              60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65              70              75              80

Tyr Leu Glu Leu Arg Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
            85              90              95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:129:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 125 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Thr Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn
            20              25              30
```

```
Phe Pro Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Lys Ile Val Asn Gly Glu Lys Lys Tyr Ser Gln Lys
    50              55              60

Phe Val Asp Arg Val Thr Phe Thr Gly Asp Thr Ser Ala Asn Thr Ala
65              70              75              80

Tyr Met Glu Val Arg Gly Leu Arg Ser Ala Asp Thr Ala Thr Tyr Tyr
                85              90              95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Met Asp Pro Gln Ala Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:130:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys
    50              55              60

Phe Arg Asp Arg Val Thr Phe Thr Ala Asp Thr Asp Ala Asn Thr Ala
65              70              75              80

Tyr Met Glu Leu Arg Ser Leu Arg Ser Ala Asp Thr Ala Ile Tyr Tyr
                85              90              95

Cys Ala Arg Val Gly Pro Tyr Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105             110
```

```
Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:131:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10                  15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25                  30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Thr Gln Asp Leu Glu Trp
            35              40                  45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Lys Glu Ile Ser Pro Lys
        50              55                  60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65                  70              75                  80

Tyr Leu Glu Leu Arg Ser Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
                85                  90                  95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105                 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:132:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 124 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

```
Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1               5               10              15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
            20              25              30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
        35              40              45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Pro Pro Lys
        50              55              60

Phe Gln Asp Arg Val Ser Leu Thr Arg Asp Thr Ser Ala Gly Thr Val
65              70              75              80

Tyr Leu Glu Leu Thr Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
            85              90              95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
            100             105             110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
        115             120
```

(2) INFORMATION FOR SEQ ID NO:133:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

TCGAGGGTCG GTCGGTCTCT AGACGGTCGG TCGGTCA   37

(2) INFORMATION FOR SEQ ID NO:134:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

CTAGTGACCG ACCGACCGTC TAGAGACCGA CCGACCC   37

(2) INFORMATION FOR SEQ ID NO:135:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

CGGTCGGTCG GTCCTCGAGG GTCGGTCGGT CT   32

(2) INFORMATION FOR SEQ ID NO:136:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 40 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

CTAGAGACCG ACCGACCCTC GAGGACCGAC CGACCGAGCT   40

210

(2) INFORMATION FOR SEQ ID NO:137:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

CAAGGAGACA GGATCCATGA AATAC   25

(2) INFORMATION FOR SEQ ID NO:138:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

AGGGCGAATT GGATCCCGGG CCCCC   25

(2) INFORMATION FOR SEQ ID NO:139:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 29 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

   (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

CTAGTCATCA TCATCATCAT TAAGCTAGC   29

(2) INFORMATION FOR SEQ ID NO:140:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 29 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

   (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

CTAGGCTAGC TTAATGATGA TGATGATGA   29

(2) INFORMATION FOR SEQ ID NO:141:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 13 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 1
        (D) OTHER INFORMATION: /label= J

    (ix) FEATURE:
        (A) NAME/KEY: Modified-site
        (B) LOCATION: 13
        (D) OTHER INFORMATION: /label= ZC

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

212

```
Ser Ile Ser Ile Gly Pro Gly Arg Ala Phe Tyr Thr Gly
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:142:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 126 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

```
Leu Leu Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu Thr Leu Ser
1               5                   10                  15

Leu Thr Cys Thr Val Ser Gly Gly Ser Leu Ser Ser Phe Asp Trp Asn
            20                  25                  30

Trp Ile Arg Gln Pro Ala Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile
        35                  40                  45

Tyr Pro Ser Gly Asn Thr His Tyr Asn Pro Ser Leu Arg Ser Arg Val
    50                  55                  60

Thr Met Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser Val Lys Leu Thr
65                  70                  75                  80

Ser Val Thr Ala Ala Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Glu Asn
            85                  90                  95

Thr Gly Arg Thr Ile Glu Glu Ile Gly Asn Phe Phe Asp Ile Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
            115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:143:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 122 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

213

```
Leu Leu Lys Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1               5                   10                  15

Leu Ser Cys Val Ile Ser Ala Phe Ser Phe Ser Gly Tyr Asn Ile Asn
              20              25              30

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile
          35              40              45

Ser Met Ser Thr Gly Ser Leu Ser Tyr Ala Asp Ser Met Lys Gly Arg
      50              55              60

Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Val Tyr Leu Glu Met
65              70              75                  80

Ser Ser Leu Thr Ala Glu Asp Thr Ala Met Tyr Tyr Cys Ala Ala Arg
              85              90              95

Thr Pro Leu Val Gly Arg Ala Leu Asp Ile Trp Gly Gln Gly Thr Val
          100             105             110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly
      115             120
```

(2) INFORMATION FOR SEQ ID NO:144:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 132 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

```
Leu Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1               5                   10                  15

Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met Asn
              20              25              30

Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Val Ala Tyr Ile
          35              40              45

Ser Ser Ser Arg Lys Tyr Thr Glu Tyr Ala Asp Ser Val Lys Gly Arg
      50              55              60

Phe Thr Ile Ser Arg Glu Asn Ala Lys Tyr Ser Val Phe Leu Gln Leu
65              70              75                  80
```

```
Asp Ser Leu Thr Ala Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Gly
            85              90              95

Arg Asp Phe Tyr Ser Gly Phe Gly Arg Arg Asp Asp Phe His Leu His
            100             105             110

Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser Ala
            115             120             125

Ser Thr Lys Gly
    130
```

(2) INFORMATION FOR SEQ ID NO:145:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 126 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

```
Leu Leu Glu Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
1               5               10              15

Arg Ile Ser Cys Val Ala Ser Gly Asp Ile Phe Tyr Ser Tyr Ala Met
            20              25              30

Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ser
        35              40              45

Ile Ser Gly Thr Gly Gly Ser Asn Tyr Tyr Ala Asp Ser Val Lys Gly
    50              55              60

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Thr Leu Tyr Leu Gln
65              70              75              80

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
            85              90              95

Asp Arg Gly Pro Arg Ile Gly Ile Arg Gly Trp Phe Asp Ser Trp Gly
            100             105             110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
        115             120             125
```

(2) INFORMATION FOR SEQ ID NO:146:

    (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 124 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: protein

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

    Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg
    1   5   10   15

    Leu Ser Cys Ala Ala Ser Gly Phe Leu Tyr Ser Ser Phe Ala Met Ser
                20   25   30

    Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Ala Trp Val Ser Thr Ile
            35   40   45

    Ser Ala Ser Gly Gly Ser Thr Lys Tyr Ala Asp Ser Val Lys Gly Arg
        50   55   60

    Phe Ile Ile Ser Arg Asp Asn Ser Lys Asn Thr Ile Tyr Leu Gln Met
    65   70   75   80

    Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asn
                85   90   95

    Phe Arg Ala Phe Ala Arg Asp Pro Trp Gly Asp Trp Gly Gln Gly Thr
                100   105   110

    Leu Val Thr Val Ser Ser Ala Ser Ala Ser Thr Lys
            115   120

(2) INFORMATION FOR SEQ ID NO:147:

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 109 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: protein

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

    Met Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
    1   5   10   15

    Glu Arg Val Ile Val Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn
                20   25   30

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```
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40              45

Ile Tyr Gly Ala Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55              60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70              75              80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Gly
                85                  90              95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
            100             105
```

(2) INFORMATION FOR SEQ ID NO:148:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 112 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

```
Met Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1               5               10              15

Glu Arg Ala Thr Phe Ser Cys Arg Ser Ser His Ser Ile His Thr Arg
            20              25              30

Arg Val Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val
        35                  40              45

Ile His Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser
    50                  55              60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Val Glu
65                  70              75              80

Pro Glu Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser
                85                  90              95

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys Arg Thr Val Val
            100             105             110
```

(2) INFORMATION FOR SEQ ID NO:149:

217

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 111 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: protein

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

Met Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1   5   10   15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Asn Gly
            20   25   30

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35   40   45

Ile Tyr Gly Ala Ser Thr Arg Ala Thr Asp Ile Pro Asp Arg Phe Ser
    50   55   60

Gly Ser Gly Ser Gly Ala Asp Phe Thr Leu Ala Ile Ser Arg Leu Glu
65   70   75   80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Ala Gly Ser His
            85   90   95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
            100   105   110

(2) INFORMATION FOR SEQ ID NO:150:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 111 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: protein

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

Met Ala Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Pro Ser Gln Gly Ile Gly Arg Phe
            20   25   30

Phe Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile

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```
        35                    40                    45
    Tyr Ala Ala Asp Ile Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
        50                    55                    60

    Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
    65                    70                    75                    80

    Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr
                      85                    90                    95

    Thr Phe Gly Gln Gly Thr Arg Leu Asp Ile Lys Arg Thr Val Ala
                  100                   105                   110
```

(2) INFORMATION FOR SEQ ID NO:151:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 112 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

```
    Met Ala Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
    1                 5                    10                    15

    Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Val Ser Ser Ser
                  20                    25                    30

    Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Val
              35                    40                    45

    Ile Phe Gly Ala Tyr Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
        50                    55                    60

    Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
    65                    70                    75                    80

    Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
                      85                    90                    95

    Ile Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala
                  100                   105                   110
```

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What Is Claimed Is:

    1.   A human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp120 and neutralizing HIV, wherein the monoclonal antibody has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 66, 67, 68, 70, 72, 73, 74, 75, 78 and 97, and conservative substitutions thereof.

    2.   The human monoclonal antibody of claim 1 wherein the monoclonal antibody has the binding specificity of a monoclonal antibody having heavy and light chain immunoglobulin variable region amino acid residue sequences in pairs selected from the group consisting of SEQ ID NOs 66:95, 67:96, 72:102, 66:97, 73:107, 74:103, 70:101, 68:98, 75:104, 72:105, 78:110, 66:118, 66:122, 66:121, 66:115, 97:124, 97:132 and 66:98, and conservative substitutions thereof.

    3.   The human monoclonal antibody of claim 1, wherein the monoclonal antibody has the binding specificity of a monoclonal antibody produced by ATCC 69078, ATCC 69079 or ATCC 69080.

    4.   The human monoclonal antibody of claim 3, wherein the monoclonal antibody is the monoclonal antibody produced by ATCC 69078, ATCC 69079 or ATCC 69080.

    5.   A human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp120 and neutralizing HIV, wherein the monoclonal antibody has the binding specificity of a monoclonal antibody comprising a light chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 95, 96, 97, 98, 101, 102, 103, 104, 105, 107, 110,

115, 118, 121, 122, 124 and 132, and conservative
substitutions thereof.

      6.   A polynucleotide sequence encoding a heavy
chain immunoglobulin variable region amino acid
5   residue sequence portion of a human monoclonal
antibody capable of immunoreacting with human
immunodeficiency virus (HIV) glycoprotein gp120 and
neutralizing HIV, wherein the monoclonal antibody has
the binding specificity of a monoclonal antibody
10   comprising said heavy chain immunoglobulin variable
region amino acid residue sequence selected from the
group consisting of SEQ ID NOs 66, 67, 68, 70, 72, 73,
74, 75, 78 and 97, and conservative substitutions of
the amino acid residue sequence, and polynucleotide
15   sequences complementary thereto.

      7.   The polynucleotide sequence of claim 6,
wherein the polynucleotide is DNA.

      8.   A polynucleotide sequence encoding a light
chain immunoglobulin variable region amino acid
20   residue sequence portion of a human monoclonal
antibody capable of immunoreacting with human
immunodeficiency virus (HIV) glycoprotein gp120 and
neutralizing HIV, wherein the monoclonal antibody has
the binding specificity of a monoclonal antibody
25   comprising said light chain immunoglobulin variable
region amino acid residue sequence selected from the
group consisting of SEQ ID NOs 95, 96, 97, 98, 101,
102, 103, 104, 105, 107, 110, 115, 118, 121, 122, 124
and 132, and conservative substitutions of the amino
30   acid residue sequence, and polynucleotide sequences
complementary thereto.

      9.   A human monoclonal antibody capable of
immunoreacting with human immunodeficiency virus (HIV)
glycoprotein gp41 and neutralizing HIV, wherein the
35   monoclonal antibody has the binding specificity of a

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monoclonal antibody comprising a heavy chain
immunoglobulin variable region amino acid residue
sequence selected from the group consisting of SEQ ID
NOs 142, 143, 144, 145 and 146, and conservative
5   substitutions thereof.

10.   The human monoclonal antibody of claim 9
wherein the monoclonal antibody has the binding
specificity of a monoclonal antibody having heavy and
light chain immunoglobulin variable region amino acid
10   residue sequences in pairs selected from the group
consisting of SEQ ID NOs 142:147, 143:148, 144:149,
145:150, and 146:151, and conservative substitutions
thereof.

11.   A human monoclonal antibody capable of
15   immunoreacting with human immunodeficiency virus (HIV)
glycoprotein gp41 and neutralizing HIV, wherein the
monoclonal antibody has the binding specificity of a
monoclonal antibody comprising a light chain
immunoglobulin variable region amino acid residue
20   sequence selected from the group consisting of SEQ ID
NOs 147, 148, 149, 150, and 151 and conservative
substitutions thereof.

12.   A polynucleotide sequence encoding a heavy
chain immunoglobulin variable region amino acid
25   residue sequence portion of a human monoclonal
antibody capable of immunoreacting with human
immunodeficiency virus (HIV) glycoprotein gp41 and
neutralizing HIV, wherein the monoclonal antibody has
the binding specificity of a monoclonal antibody
30   comprising said heavy chain immunoglobulin variable
region amino acid residue sequence selected from the
group consisting of SEQ ID NOs 142, 143, 144, 145, and
146, and conservative substitutions of the amino acid
residue sequence, and polynucleotide sequences
35   complementary thereto.

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13. The polynucleotide sequence of claim 12, wherein the polynucleotide is DNA.

14. A polynucleotide sequence encoding a light chain immunoglobulin variable region amino acid residue sequence portion of a human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp41 and neutralizing HIV, wherein the monoclonal antibody has the binding specificity of a monoclonal antibody comprising said light chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID NOs 147, 148, 149, 150, and 151, and conservative substitutions of the amino acid residue sequence, and polynucleotide sequences complementary thereto.

15. A host cell comprising the polynucleotide sequence of claim 6, 8, 12 or 14.

16. A DNA expression vector comprising the polynucleotide sequence of claim 6, 8, 12 or 14.

17. A method of detecting human immunodeficiency virus (HIV) comprising contacting a sample suspected of containing HIV with a diagnostically effective amount of the monoclonal antibody of claim 1, 5, 9 or 11 and determining whether the monoclonal antibody immunoreacts with the sample.

18. The method of claim 17, wherein the detecting is in vivo.

19. The method of claim 18, wherein the monoclonal antibody is detectably labelled with a label selected from the group consisting of a radioisotope and a paramagnetic label.

20. The method of claim 17, wherein the detecting is in vitro.

21. The method of claim 20, wherein the monoclonal antibody is detectably labelled with a

label selected from the group consisting of a
radioisotope, a fluorescent compound, a colloidal
metal, a chemiluminescent compound, a bioluminescent
compound, and an enzyme.

5   22.   The method of claim 20, wherein the
monoclonal antibody is bound to a solid phase.

           23.   A method for providing passive immunotherapy
to human immunodeficiency virus (HIV) disease in a
human, comprising administering to the human an

10   immunotherapeutically effective amount of the
monoclonal antibody of claim 1, 5, 9 or 11.

           24.   The method of claim 23, wherein the passive
immunotherapy is provided prophylactically.

           25.   The method of claim 23, wherein the

15   administering is parenteral administration.

           26.   The method of claim 25, wherein the
parenteral administration is by subcutaneous,
intramuscular, intraperitoneal, intracavity,
transdermal, or intravenous injection.

20   27.   The method of claim 25, wherein the
parenteral administration is by gradual perfusion.

           28.   The method of claim 27, wherein the gradual
perfusion is by intravenous or peristaltic means.

           29.   The method of claim 25, wherein the

25   immunotherapeutically effective amount is from about
0.1 mg/kg to about 300 mg/kg.

           30.   A method for inducing active immunotherapy
to human immunodeficiency virus (HIV) disease in a
human which comprises administering to the human an

30   immunogenically effective amount of an anti-idiotype
antibody to the monoclonal antibody of claim 1, 5, 9
or 11.

           31.   A pharmaceutical composition comprising at
least one dose of an immunotherapeutically effective

35   amount of the monoclonal antibody of claim 1, 5, 9 or

11 in a pharmacological carrier.

32. The pharmaceutical composition of claim 31 wherein said composition contains two or more different monoclonal antibodies.

33. A kit useful for the detection of human immunodeficiency virus (HIV) in a source suspected of containing HIV, the kit comprising carrier means being compartmentalized to receive in close confinement therein one or more containers comprising a container containing the monoclonal antibody of claim 1, 5, 9 or 11.

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SHINE-DALGARNO MET

GGCCGCAAATTCTATTTCAAGGAGACAGTCATAATG
CGTTTAAGATAAAGTTCCTCTGTCAGTATTAC

LEADER SEQUENCE

AAATACCTATTGCCTACGGCAGCCGCT
TTTATGGATAACGGATGCCGTCGGCGA

LEADER SEQUENCE

GGATTGTTATTACTCGCTGCCCAACCAG
CCTAACAATAATGAGCGACGGGTTGGTC

LINKER   LINKER

| NCOI | V_H BACKBONE | XHOI | SPEI |

CCATGGCCCAGGTGAAACTGCTCGAGATTTCTAGACTAGT
GGTACCGGGTCCACTTTGACGAGCTCTAAAGATCTGATCA

STOP   LINKER

TyrProTyrAspValProAspTyrAlaSer
TACCCGTACGACGTTCCGGACTACGGTTCTTAATAGAATTCG
ATGGGCATGCTGCAAGGCCTGATGCCAAGAATTATCTTAAGCAGCT

FIGURE 1
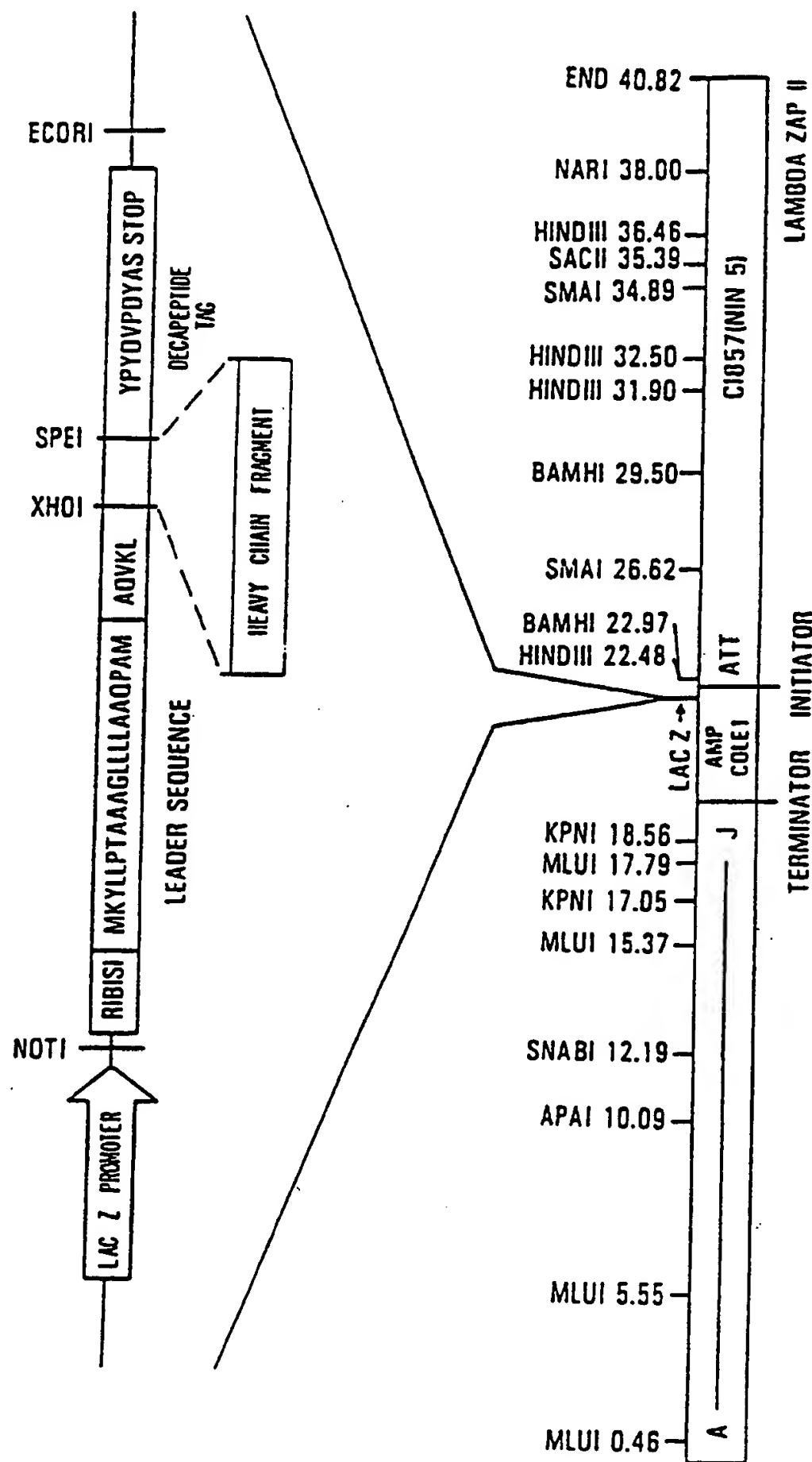
FIGURE 2

ECOR I   SHINE-DALGARNO   MET

TGAATTCTAAACTAGTCGCCAAGGAGACAGTCATAATGAAAT
TCGAACTTAAGATTGATCAGCGGTTCCTCTGTCAGTATTACTTTA

LEADER SEQUENCE

ACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAG
TGGATAACGGATGCCGTCGGCGACCTAACAATAATGAGCGACGGGTTGGTC

NCO I   SAC I   XBA I   Not I

CCATGGCCGAGCTCGTCAGTTCTAGAGTTAAGCGGCCG
GGTACCGGCTCGAGCAGTCAAGATCTCAATTCGCCGGCAGCT
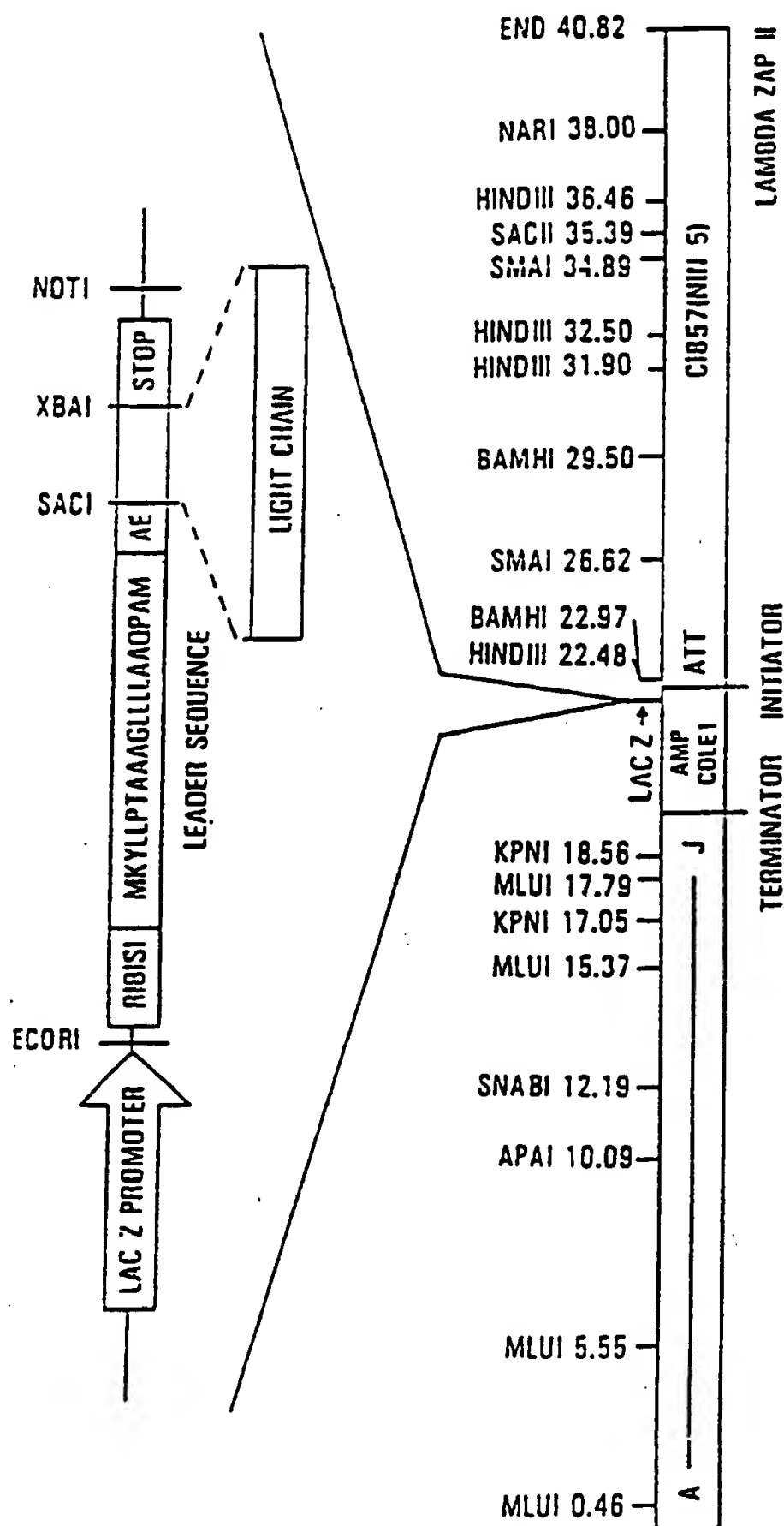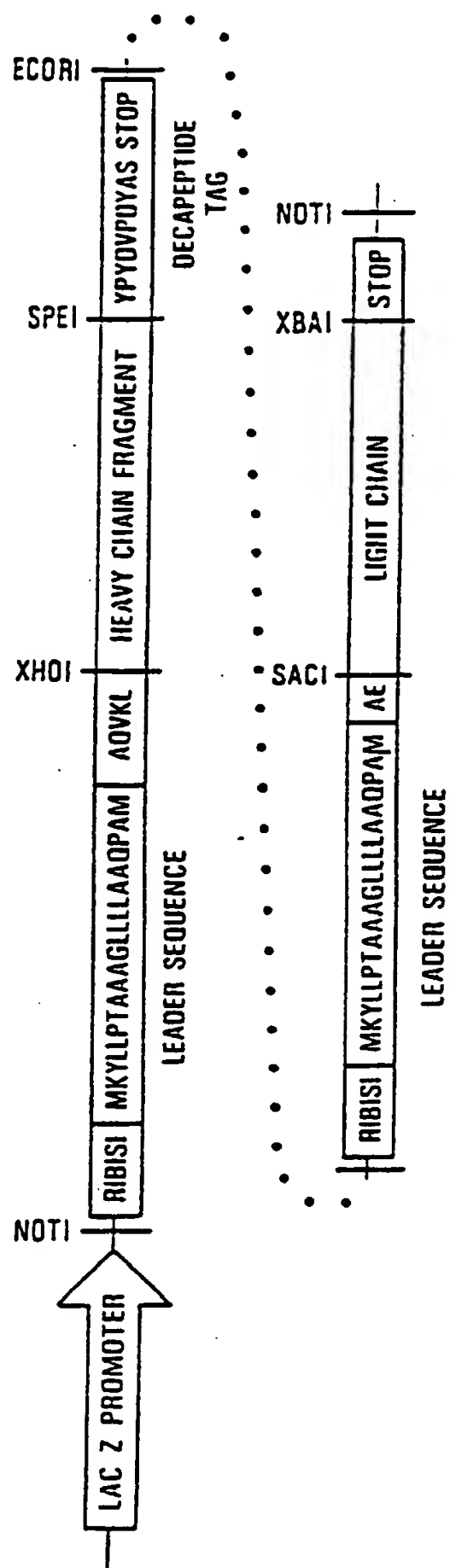
FIGURE 3

FIGURE 4

FIGURE 5

Neutralization of HIV-1 by Recombinant Fabs

clone no.	Fab conct (µg/ml)	ELISA titer	p24 assay MN	p24 assay IIIB	syncytia assay MN
1	1.8	1:8	-	-	-
2	3.1	1:64	-	-	-
3	4.1	1:32	-	-	-
4	25.0	1:16	40	80	>128
5	2.4	1:128	-	-	-
6	4.0	1:64	-	-	-
7	4.5	1:64	20	20	32
8	14.0	1:256	20	20	-
11	11.0	1:128	-	-	-
12	6.0	1:64	80	40	>128
13	6.1	1:128	80	80	-
18	0.9	1:128	-	20	-
20	6.9	1:256	-	-	32
21	8.5	1:32	20	20	32
22	8.6	1:64	20	20	-
24	0.7	1:32	-	-	-
27	10.0	1:64	20	20	32
29	16.0	1:1024	-	-	-
31	9.3	1:128	-	-	-
35	8.9	1:64	-	-	-
2F5mAb	10.0		40	160	
2F5Fab	5.0		40	20	
F58mAb	10.0		160	40	
F58F(ab')$_2$	200.0		40	20	

FIGURE 6
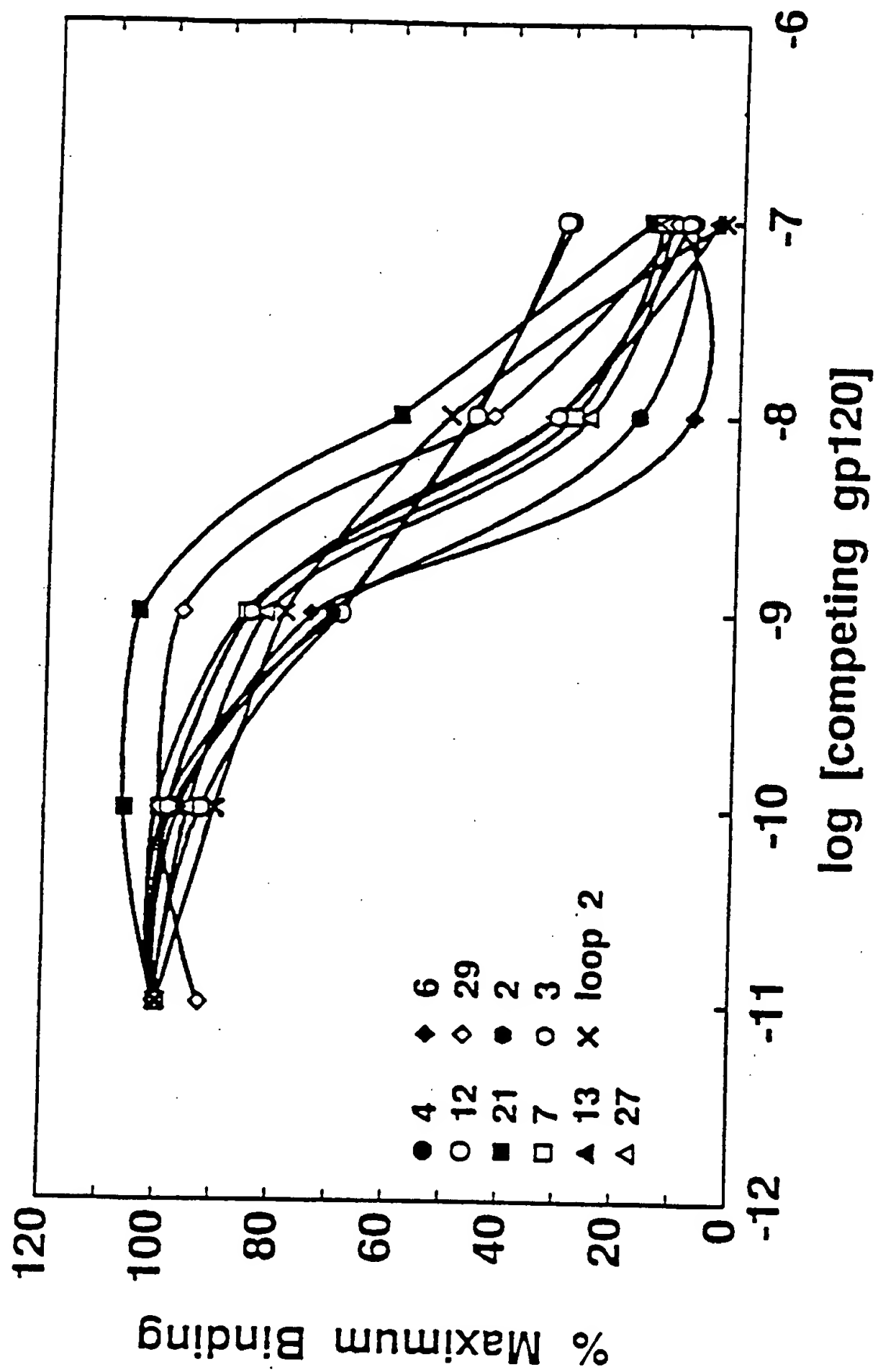
FIGURE 7
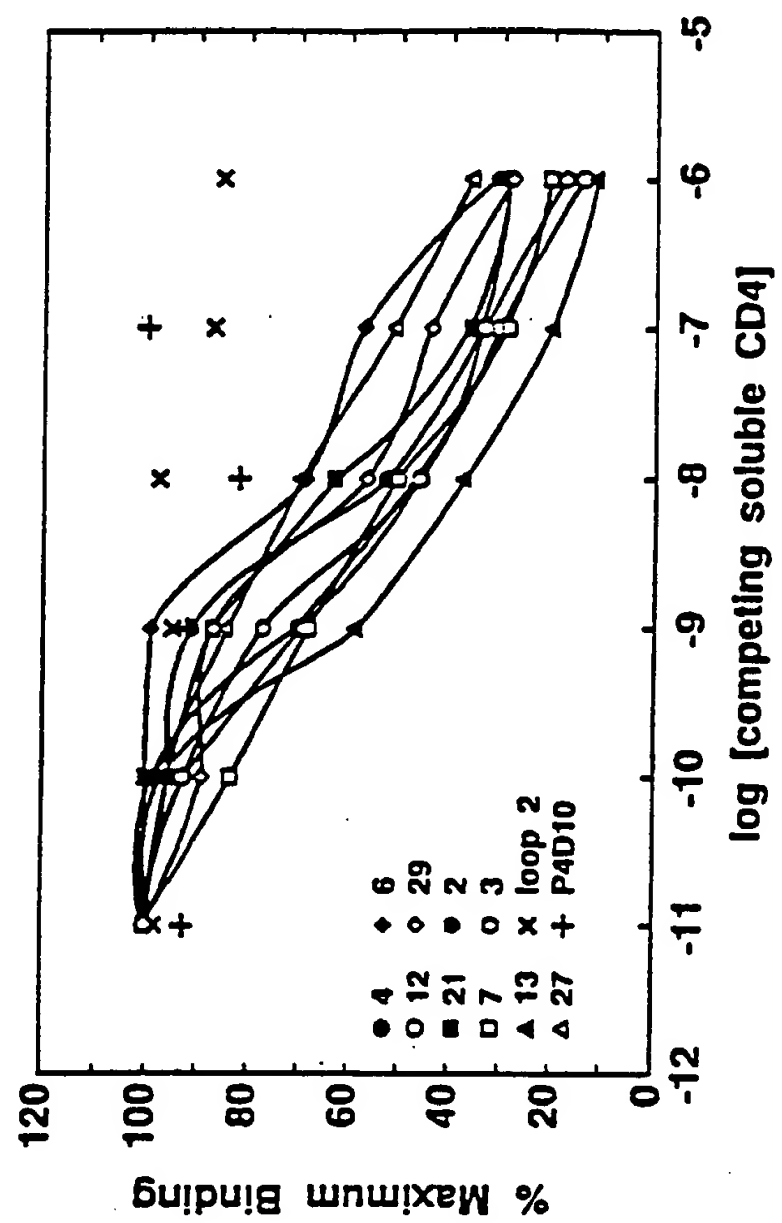
FIGURE 8
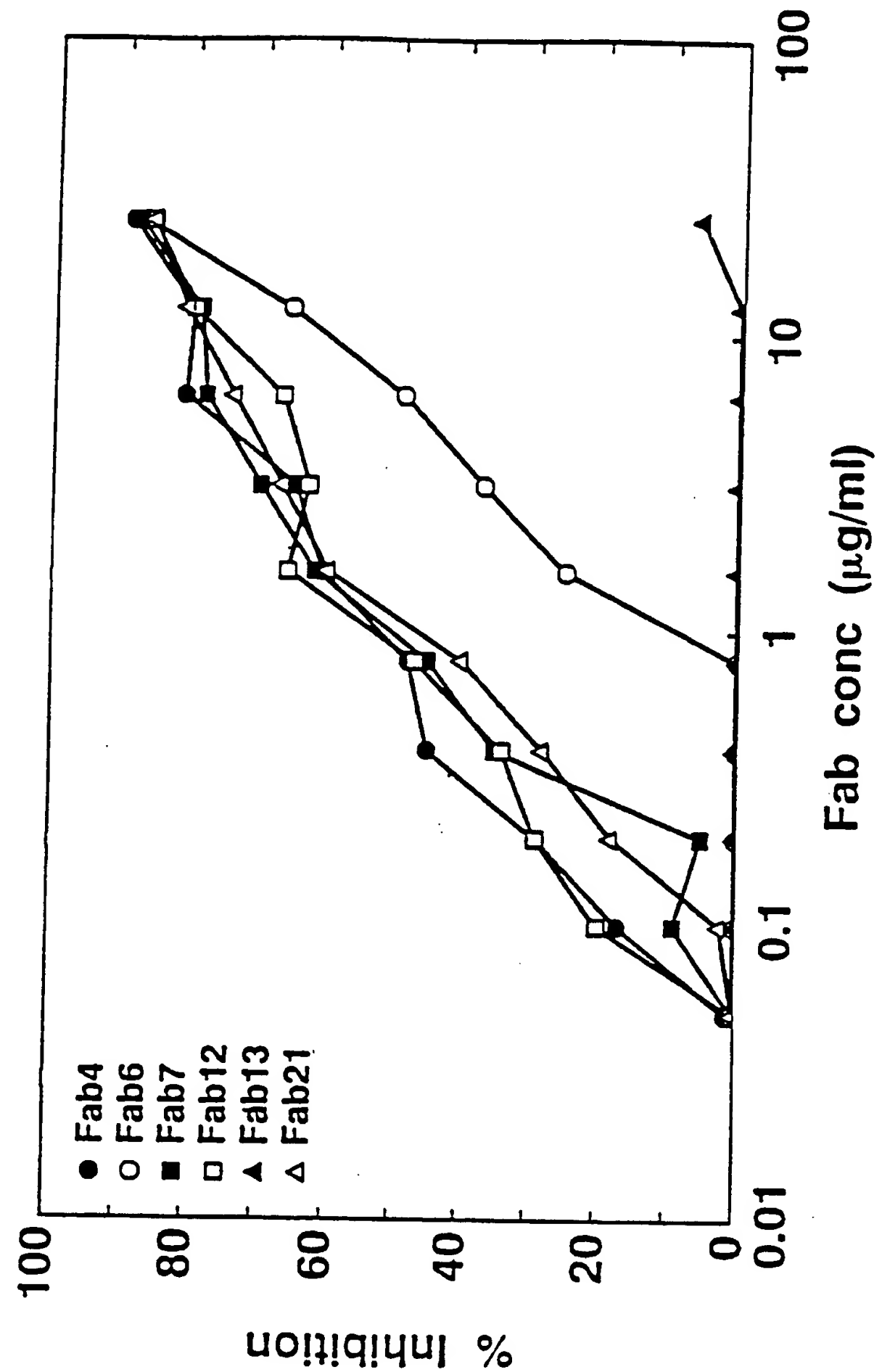
FIGURE 9

Alignment of gp120 binders (V$_H$)

FIGURE 10

FIGURE 11

III2-LCn shuffled chains

Clone	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	SEQ ID NO
IIIV-III2/L12	ELTQAPGTLSLSPGERATFSC	RSSHSIRSRRVR	WYQHRPGQAPRLVIH	GVSNRAS	GISDRFSGSGSGTDFTLTITRVEPEDFALYYC	QVYGASSYT	FGQGTRLERKR	97
IIIV-III2/LC11	...S......D......	...N.....A	....	.......F	....	....	......DF...	114
IIIV-III2/LC24	...S......D......	...N.....A	...........L...	.......F	....	....	......DF...	113
IIIV-III2/LC22	...S.............	...N.....A	...........L...	.......F	....	....	......DF...	115
IIIV-III2/LC1	...S.D....NV...L.	..A..R.S...LA	....R......L.Y	..S..G	.VP......M....	.T..G.....	.....VDI...	116
IIIV-III2/LC7	...S.D....NA...L.	..A..R.S...LA	....R......L.Y	.....G	.VP......S..S.L..	.T..G.....	.....TVDI..	117
IIIV-III2/LC20	...S.D....NT...L.	..A..R.G...LA	..RR......L.Y	.....G	.VP......S..S.L..	.T..G.....	.....VDI...	118
IIIV-III2/LC13	...S......T....IL.	RT..N.N...LA	...L.S......L.Y	..N..G	..P.......V.....	.T..G.A...	......DI...	119
IIIV-III2/LC3	...S......T....IL.	RT..N.N...LA	...L.S......L.Y	..N..G	..P.......V.....	.T..G.A...	......DI...	120
IIIV-III2/LC5	...S......T......	RT..N.N...LA	...L.S......L.Y	..N..G	..P.......V.....	.T..G.A...	....	
IIIV-III2/LC26	...S......ST....IL.	RT..N.N...LA	...V.S.LP...L..	..R..G	..P......S.L.A...V...	.T..G...S	......DFW.	121
IIIV-III2/LC25	...S.....M.....VL.	.T.RN.N...LA	...VRR......L..	..N..G	.VP......S.L......V.F.	.T..G.....	...M.DIR.	122
IIIV-III2/L12	ELTQAPGTLSLSPGERATFSC	RSSHSIRSRRVR	WYQIHRPGQAPRLVIH	GVSNRAS	GISDRFSGSGSGTDFTLTITRVEPEDFALYYC	QVYGASSYT	FGQGTRLERKR	97

FIGURE 12

FIGURE 13

FIGURE 14B

FIGURE 14A

tac promoter

                       rbs   BamHI   pelB leader

                                      MetLysTyrLeuLeuProThrAlaAlaAlaGly

TG[TGACAATTAATCATCGGCTCGTATAAT]GTGTGGAATTGTGAGCGGATAACAATTTCACA[AGGAGG]AGGATCCATGAAATACCTATTGCCTACGGCAGCCGCTGGA
AC[ACTGTTAATTAGTAGCCGAGCATATT]CACACCTTAACACTCGCCTATTGTTAAAGTGTC[TCCTCC]TCCTAGGTACTTTATGGATAACGGATGCCGTCGGCGACCT

SacI   XhoI   XbaI
                                      STOP

LeuLeuLeuAlaAlaGlnProAlaMetAlaGluLeu
TTGTTATTACTCGCTGCCCAACCAGCCATGGCCGAGCTCGGTCGGTCGGTCGGTCTCTGAGGGTCGGTCGGTCGGTCCCGC
AACAATAATGAGCGACGGGTTGGTCGGTACCGGCTCGAGCTCCCAGCCAGGAGCTCCCAGCCAGAGATCTCAATTCGCCGGCG

FIGURE 15A

tac promoter

                       rbs   BamHI   pelB leader

                                      MetLysTyrLeuLeuProThrAlaAlaAlaGly

TG[TGACAATTAATCATCGGCTCGTATAA]GTGTGGAATTGTGAGCGGATAACAATTTCACA[AGGAGG]AGGATCCATGAAATACCTATTGCCTACGGCAGCCGCTGGA
AC[ACTGTTAATTAGTAGCCGAGCATATT]CACACCTTAACACTCGCCTATTGTTAAAGTGTC[TCCTCC]TCCTAGGTACTTTATGGATAACGGATGCCGTCGGCGACCT

     XhoI   XbaI   SpeI   (His)₆-tail
                                   ThrSerHisHisHisHisHisHisSTOP

LeuLeuLeuAlaAlaGlnProAlaMetAlaGlnValLysLeuLeuGlu
TTGTTATTACTCGCTGCCCAACCAGCCATGGCCGCCCAGTGAAACTGCTCGAGGGTCGGTCGGTCTCTAGACGGTCGGTCGGTCTCTAGACGGTCGGTCGGTCGGTCGGTCGGTCGGTCTCTTAAGCTA
AACAATAATGAGCGACGGGTTGGTCGGTACCGGGTCCACTTTGACGAGCTCCCAGCCAGGATCTGCCCAGCCAGTGATCAGTCAGTAGTAGTAGTAATTCGAT

FIGURE 15B

	b11	b6	b4	b12	b7	b21	b3	b13	b22	B26	b8	b18	b27	B8	B35	s4	b1	b14	b24	s8	p35
b11	+	+	w	+	+	−	+	+	−	−	+	−	−	−	−	−	+	−	−	−	−
b6	+	+	+	+	+	−	+	+	−	−	+	−	−	−	−	−	+	+	+	−	−
b4	+	+	+	+	+	+	+	+	−	−	+						−	−	−	−	−
b12	+	+	+	+	+	+	+	+			+										
b7	+	+	+	+	+	+	+	+	−		+						w	−	−	w	~
b21	+	+	+	+	+	+	+	−	−	−	+						+	−	−	−	−
b3	+	+	−	+	+	−	+	+	−	−	+						−	−	−	−	−
b13	w	w	−	+	+	−	−	+	+	+	+	+	+	+	+	+	−	−	−	w	−
b22	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
B26	w	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
b8	+	+	+	+	+	−	w	+	+	+	+	+	+	+	+	+	−	−	−	−	−
b18	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	w	−	−
b27	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
B8	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
B35	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	w	−	−
s4	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
b1	+	+	+	+	+	−	−	+	w	−	+	w	−	−	−	−	+	+	+	−	−
b14	w	+	+	+	+	w	−	+	−	−	+	−	−	−	−	−	+	+	+	−	−
b24	+	+	+	+	+	−	+	−	−	−	+	−	−	−	−	−	+	+	+	−	−
s8	w	+	+	+	+	−	w	+	+	w	+	+	+	+	+	+	−	−	−	+	−
p35	+	+	+	+	+	−	+	w	w	w	+	w	w	w	w	+	w	−	−	−	+

FIGURE 16
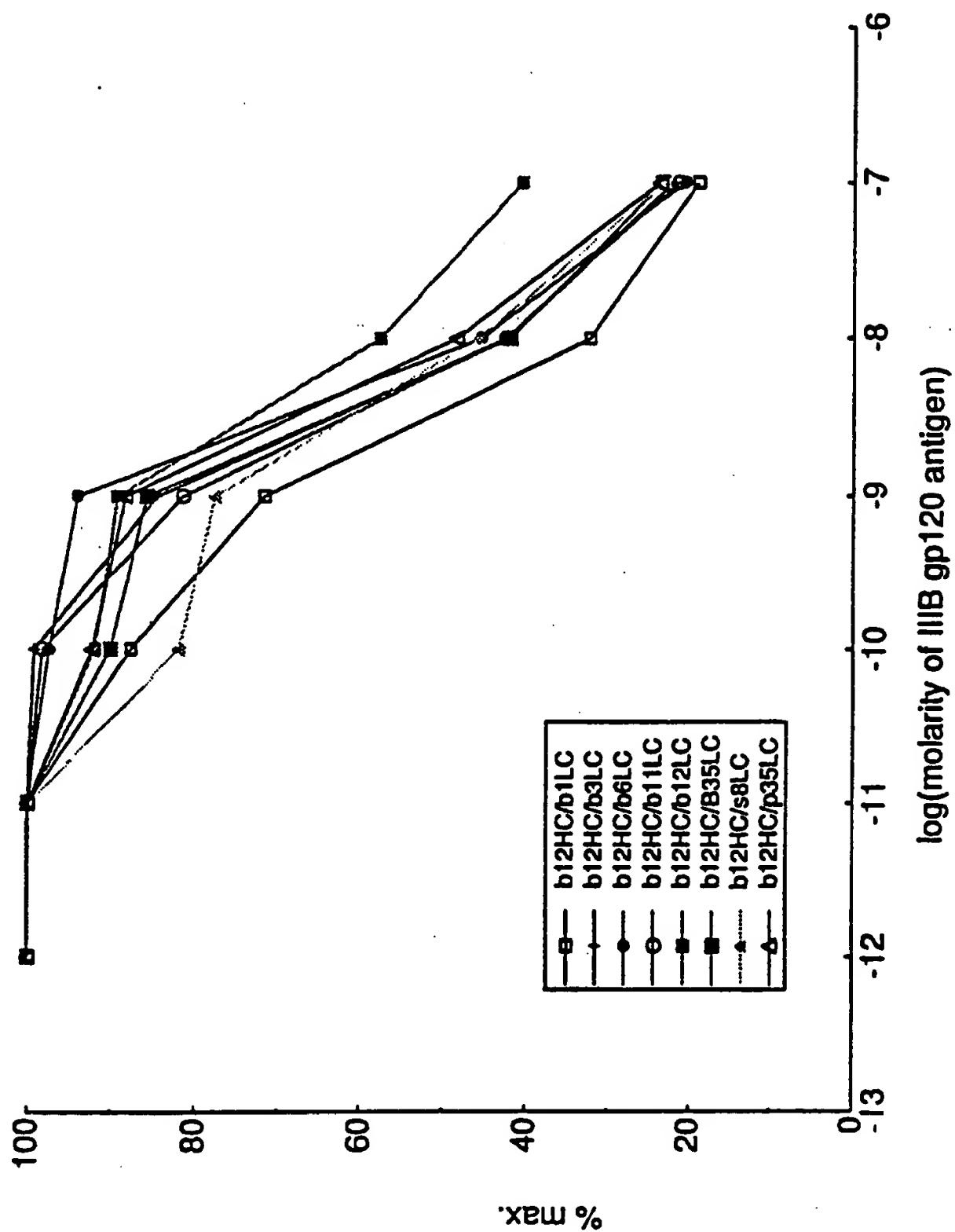
FIGURE 17

GF41 BINDERS: HEAVY CHAIN SEQUENCES

CLONE	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
DL 41 19	LLESGFGLVKFSETLSLTCTVSGGSLS	SFDMN	WIRQPKGKGLEWIG	RIYPSGNTNYNPSLRS	RVTHSRDTSKNQFSVKLTSVTAADTALYYCAR	ENTGRTIEEIGNFFDI	WGQGTLVTVSSASTKG
DO 41 11	LLKSGGGLVKTGGSLRLSCVISAFSFS	GYNIN	WVRQAPGKGLEWVS	SISKSTDSLSTADSNKG	RFTISRDHARHSVTLEHSSLTAEDTAMYYCAA	RTPLVGRALDI	WGQGTVVTVSSASTKG
GL 41 1	LLESGGGLVKFGGSLRLSCSASGFTFS	SYGMN	WVRQAPGKGPEWVA	YISSSRKYTEYADSVKG	RFTISREHAKYSVFLQLDSLTAEDTAIYYCAR	GRDFYSGFGRRDDFHLAYHDV	WGKGTTVTVSSASTKG
HT 41 12	LLEQSGGGLVQPGGSLRISCVASGDIFY	SYAMS	WVRQAPGKGLEWVA	SISGTGGSNYYADSVKG	RFTISRDHSKSTLYLQHNSLRAEDTALYYCAR	DRGFRIGIRGWFDS	WGQGTLVTVSSASTKG
SS 41 8	LLESGGGLVQPGGSLRLSCAASGFLVS	SFAMS	WVRQANGKGLAWVS	TISASGGSTKYADSVKG	RFIISRDHSKNTIYLQHDSLRAEDTAVYYCAK	NFRAFARDYWGD	WGQGTLVTVSSASASTK

FIGURE 18

GP41 BINDERS: LIGHT CHAIN SEQUENCES

CLONE	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
DL 41 19	HAELTQSPGTLSLSPGERVIVSC	RASQSVSSNYLA	WYQQKPGQAPRLLIY	GASNRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSQT	FGQGTKVEIKRT
DO 41 11	HAELTQSPGTLSLSPGERATFSC	RSSHSIHTRRVA	WYQIIKPGQAPRLVIH	GVSNRAS	GISDRFSGSGSGTDFTLTIRVEPEDFALYYC	QVYGASSYT	FGQGTRLERKRTVV
GL 41 1	HAELTQSPGTLSLSPGERATLSC	RASQSVSIGYLA	WYQKPGQAPRLLIY	GASTRAT	DIPDRFSGSGSGADFTLAISRLEPEDFAVYYC	QQYAGSHT	FGQGTKLEIKRTVA
HT 41 12	HAELTQSPSSLSASVGDRVTITC	RPSQGIGRFFN	WYQQKYGKAPNLLIY	AADILQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPYT	FGQGTRLDIKRTVA
SS 41 8	HAELTQSPSSLSASVGDRVTITC	RASQGVSSSYLA	WYQQKPGQAPRLVIF	GAVSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSPIT	FGQGTKVDIKRTVA

FIGURE 19

INTERNATIONAL SEARCH REPORT

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :C07K 15/28; A61K 39/395, 43/00
US CL   :424/1.1, 85.8, 86; 530/388.15;

According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   424/1.1, 85.8, 86; 435/5, 7.92, 69.6, 70.21, 172.2, 172.3, 240.27, 252.3, 252.33, 320.1; 530/387.3, 388.15, 388.35.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

U.S. AUTOMATED PATENT SYSTEM (FILE USPAT, 1971-present).

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A., Vol. 88, issued November 1991, Burton et al., "A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive inidividuals," pages 10134-10137, see entire document and especially Figure 1.	1-5, 31-33 ---------- 17-19
Y	JOURNAL OF VIROLOGY, Vol. 65, No. 1, issued January 1991, Ho et al., "Conformational Epitope on gp120 Important in CD4 Binding and Human Immunodeficiency Virus Type 1 Neutralization Identified by a Human Monoclonal Antibody," pages 489-493, see entire document.	1-5,17-19, 31-33

[X]   Further documents are listed in the continuation of Box C.   [ ]   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 December 1993	0 5 JAN 1994

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer ROBERT D. BUDENS
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A., Vol. 88, issued April 1991, Gorny et al., "Production of site-selected neutralizing human monoclonal antibodies against the third variable domain of the human immunodeficieny virus type 1 envelope glycoprotein," pages 3238-3242, see entire document.	1-5,17-19, 31-33
Y	JOURNAL OF IMMUNOLOGY, Vol. 146, No. 12, issued 15 June 1991, Posner et al., "An IgG Human Monoclonal Antibody That Reacts with HIV-1/gp120, Inhibits Virus Bidning to Cells, and Neutralizes Infection," pages 4325-4332, see entire document.	1-5,17-19, 31-33
Y	RESEARCH VIROLOGY, Vol. 142, issued 1991, Tilley et al., "A human monoclonal antibody against CD4-binding site of HIV1 gp120 exhibits potent, broadly neutralizing activity," pages 247-259, see entire document.	1-5,17-19, 31-33
Y	AIDS RESEARCH AND HUMAN RETROVIRUSES, Vol. 8, No. 6, issued June 1992, Karwowska et al., "Production of Human Monoclonal Antibodies Specific for Conformational and Linear Non-V3 Epitopes of gp120," pages 1099-1106, see entire document.	1-5,17-19, 31-33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/09328

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
   (Telephone Practice)
   Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-5, 17-19, 31-33

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I. Claims 1-5, 17-19 and 31-33, drawn to human monoclonal antibodies to HIV gp120 and a first method of use for in vivo detection of HIV, classified in Class 530, subclass 388.15 and Class 424, subclass 1.1.

II. Claims 6-8 and 15-16, drawn to a second product, polynucleotides encoding anti-gp120 antibodies, vectors and host cells, classified in Class 536, subclass 23.53 and Class 435, subclasses 320.1 and 252.3.

III. Claims 9-11, drawn to a third product, human monoclonal antibodies to HIV gp41, classified in Class 530, subclass 388.15.

IV. Claims 12-16, drawn to a fourth product, polynucleotides encoding anti-gp41 human monoclonal antibodies, classified in Class 536, subclass 23.53.

V. Claims 20-22, drawn to a second method of use, a method for in vitro detection of HIV, classified in Class 435, subclass 5.

VI. Claims 23-29, drawn to a third method of use, a method of passive immunotherapy, classified in Class 424, subclass 86.

VII. Claim 30, drawn to a fourth method of use, a method for active immunization using anti-idiotypic antibodies, classified in Class 424, subclass 85.8.

Groups I-IV are directed to distinct products differing in their physical, chemical and immunological properties and are not so linked by a special technical feature within the meaning of PCT Rule 13.2 as to form a single general inventive concept.

Groups I and V-VII are directed to distinct methods differing in their individual method steps, reagents and utility and are not so linked by a special technical feature within the meaning of PCT Rule 13.2 as to form a single general inventive concept.

The products and method of Groups (I and III) and the methods of Groups V-VII are related as products and methods of use. The products can be used for several different methods as evidenced by Applicant's claims and the products and method of Groups (I and III) and the methods of Groups V-VII are not so linked by a special technical feature within the meaning of PCT Rule 13.2 as to form a single general inventive concept.